Presenter: Arbelaez, Carlos

Essential role of IL-23 for IL-17 and IFN-[g]-producing T helper cells

Carlos Arbelaez, Rebekka Duhen, Simon Glatigny, Tiffany Blair, Mohamed Oukka and Estelle Bettelli

University of Washington

In contrast to the established dogma of committed T cell lineages, recent evidence suggests that Th17 cells are more plastic than other T cell subsets and can acquire characteristics of Th1 cells. Both T helper subsets are involved in the pathogenesis of many autoimmune diseases. The role of the proinflammatory cytokine IL-23 and its receptor in disease pathogenesis has been studied extensively leading to conflicting reports. To directly address the role of IL-23 in the maintenance of Th17 cells we used an IL-23R GFP knockin mouse. We found that IL-23 significantly enhanced IL-17 production in WT as compared to IL-23R-deficient cells. Th1 skewed Th17 cells retained the ability to respond to IL-23 as revealed by high IL-23R expression, although the majority produced IFN-? instead of IL-17. Importantly, IL-23 reverted the phenotype in WT but not IL-23R-deficient cells. Interestingly, Th17 cells gave rise to a population of IFN-? IL-17 double producing cells in vitro in presence of IL-23, that were absent in IL-23R deficient cells. Also, after immunization, we observed a significant reduction of Th17 and IFN-?+ IL-17+ cells in vivo in IL-23R-/- mice as compared to WT controls. Furthermore, we were able to demonstrate that the acquisition of IFN-? by those cells is independent of T-bet, Eomes, STAT1 and STAT4, transcription factors and mediators of Th1 polarization, but also independent of RORgt, one of the main targets of IL-23 in Th17 cells. Together, our results confirm the important role of IL-23 in Th17 expansion, maintenance and plasticity, arguing against an end-point conversion of Th17 cells under Th1 polarizing conditions. Additionally, they point towards a novel role of IL-23 in the generation of IFN-?+ IL-17+ cells not only in vitro, but also in vivo under inflammatory conditions.

Presenter: Baldeon, Gabriela

Dysregulated T cell homeostasis in B10.A/Cr mice correlates with decreased T helper function

Gabriela Baldeon, Scott Connors, Luke Heil, Sophia Sarafova

Davidson College

The mechanisms regulating peripheral T cell homeostasis are not completely understood. During routine screening we discovered that the B10.A/Cr mouse strain in our facility has a reduced frequency of CD4 T cells in the periphery, resulting in a reduced and often inverted CD4:CD8 ratio, indicative of dysregulated T cell homeostasis. We confirmed that this is a stable and heritable phenotype by performing extensive statistical analysis to compare peripheral blood CD4:CD8 ratios between B10.A/Cr, C57Bl/10J, A/J mice, and their F1 crosses, as well as the B10.A/J strain. The comparison revealed that the B10.A/Cr mice have a significantly lower CD4:CD8 ratio than any other strain, including B10.A/J, to which they are expected to be identical. Furthermore, the homeostatic defect was due to both a decrease in naive CD4 T cells and increase in memory CD8 T cells in the lymph node, while in the spleen only a decrease in the CD4 T cells could be observed. Although in vivo and in vitro proliferation, the ability to respond to IL-7 signals, and survival capacity of the T cells were normal, we detected a small but reproducible defect in secondary immune responses. The number of IFN-?-producing CD4 T cells and the amount of IFN-? produced by both CD4 and CD8 T cells was significantly reduced in B10.A/Cr mice, as was the amount of antigen-specific IgG produced in the secondary response. Therefore, we think that the B10.A/Cr mice have diverged from the B10.A/J mice over the past sixty years and now present an attractive model for the study of T cell homeostasis.

Presenter: Barnett, Lisa

The Requirement for Antigen Presentation by Dendritic cells and B cells in Follicular helper T cell Differentiation

Lisa Barnett, Radhika Goenka, Michael P. Cancro, Mark J. Shlomchik, Gregory F. Wu, and Terri M. Laufer
University of Pennsylvania

Follicular helper T cells (TFH) are a CD4+ helper T cell subset that provide help to B cells in the germinal center, a specialized environment in which activated B cells undergo class switching and somatic hypermutation to generate high affinity antibody. We and others have recently shown that TFH differentiation initiates early after T cell activation and is independent of cognate interactions with B cells. Consistent with this, MHC class II expression restricted to CD11chi dendritic cells (DCs) drives differentiation of a TFH intermediate (termed pre-TFH). Pre-TFH express CXCR5, ICOS and the critical transcription factor Bcl-6; however, they do not secrete IL-21 and IL-4. Based on these data, we hypothesize that MHCII-positive DCs and B cells cooperate in a multi-step process to regulate TFH differentiation and that each APC subset imparts unique signals to the CD4+ T cell. To test this, we have developed several mouse models in which MHCII expression is restricted to DCs and/or B cells and B cell MHCII expression can be temporally controlled. Using these models, we find that B cell restricted antigen presentation alone is insufficient for CD4+ T cell priming and TFH formation. However, DC and B cell restricted antigen presentation combine to generate fully differentiated TFH. This suggests that DCs must initiate TFH differentiation, which is then completed by B cell antigen presentation. The systems we have developed will permit us to dissect the unique contributions of DCs and B cells to TFH differentiation.

Presenter: Barnett, Burton E.

Asymmetric B cell division in the generation of humoral immunity.

Burton E. Barnett, Maria L. Ciocca, Radhika Goenka, Lisa G. Barnett, Junmin Wu, Janis K. Burkhardt, Terri M. Laufer, Michael P. Cancro, Steven L. Reiner

Perelman School of Medicine at the University of Pennsylvania, Department of Pediatrics of Columbia University's College of Physicians and Surgeons

B cells that encode high-affinity, protective antibodies are generated in the germinal center (GC) reaction, a microanatomical structure that includes GC B cells and follicular helper T cells (TFH). GCs are seeded by a small number of antigen specific B cells that compete for selective signals. The selection of GC B cells to proliferate and differentiate into plasma cells and memory B cells relies on prolonged, motile contacts with TFH. In other instances where cells undergo prolonged external contacts, polarity cues are imparted that lead to asymmetric division. We hypothesized TFH may provide polarity cues during these interactions, in addition to mitogenic and differentiative signals, so that GC B cells may divide asymmetrically to generate diversity. Using confocal microscopy we observe that GC B cells asymmetrically segregate and unequally inherit the ancestral polarity regulator PKC?, and the receptor for interleukin 21 (IL-21R) and Bcl6, which are responsible for initiating and maintaining the GC B cell fate, respectively. We observe that neither B cells undergoing homeostatic proliferation nor GC B cells from mice deficient in leukocyte adhesion, ICAM-1-/- mice, divide asymmetrically, suggesting that cell-to-cell adhesions are responsible for initiating polarity. These adhesions may be occurring between a B cell and a T cell, as ligation of CD40 in vitro results in a high frequency of asymmetric divisions. Further examination of ICAM-1-/- mice revealed that while there is only a moderate reduction in TFH and GC B cells, the number of antibody secreting cells is severely diminished compared to wild-type mice. Together, these data support a model where, in addition to canonical signals, GC B cells receive polarity cues from TFH that result in the unequal inheritance of fate determinants by daughter B cells, leading to divergent differentiation.

Presenter: Barrigan, Lydia

Mutation in non-essential chaperone protein clpB attenuates Francisella tularensis yet induces a protective adaptive immune response

Lydia Barrigan, Lydia Barrigan, Shraddha Tuladhar, Jason Burton, Lynn Edde, Matthew Woolard, Tom Kawula, and Jeffrey Frelinger

University of North Carolina

Mutation in non-essential chaperone protein clpB attenuates Francisella tularensis yet induces a protective adaptive immune response Lydia Barrigan 1,2, Shraddha Tuladhar 2, Jason Burton 1, Lynn Edde 2, Matthew Woolard 3, Tom Kawula 1, and Jeffrey Frelinger 2. 1 Department of Microbiology and Immunology, University of North Carolina at Chapel Hill 2 Department of Immunobiology, University of Arizona 3 Department of Microbiology and Immunology, Louisiana State University Health Sciences Center Francisella tularensis is a facultative, intracellular coccobacillus and the causative agent of tularemia. F. tularensis utilizes a variety of strategies to evade the hosts immune response. One evasion mechanism is the induction of prostaglandin E2 (PGE2) by F. tularensis in infected cells. We have previously shown that production of PGE2 in the lung following intranasal F. tularensis LVS infection dampens the IFN-gamma mediated T cell response and leads to prolonged bacteremia. We therefore hypothesized that a F. tularensis mutant that failed to induce PGE2 but still was capable of intracellular growth would induce a robust immune response that would be protective against lethal LVS challenge. To test this hypothesis, we selected two F. tularensis LVS mutants that fail to induce PGE2- ?0119 and ?clpB. Of these mutants, only ?clpB is capable of intracellular growth. We tested both mutants in a pneumonic model of tularemia and found that both ?0119 and ?clpB are attenuated compared to wild-type infection. Despite rapid clearance of ?clpB from the host, this mutant induces a robust adaptive immune response similar in magnitude to an LVS infection but with increased IFN-gamma production. Although ?0119 infects macrophages in vitro, it fails to grow. Therefore, it was not surprising to find this strain was cleared rapidly from the host and did not elicit an F. tularensis-specific immune response. We next tested both mutants for their ability to protect against a lethal LVS challenge. Mice vaccinated with ?0119 rapidly succumbed to lethal LVS challenge while ?clpB protected 100% of mice. These results indicate that deletion of non-essential chaperone protein(s), like F. tularensis clpB, involved in bacterial immune evasion may serve as useful targets for future attenuated vaccine development.

Presenter: Bekiaris, Vasileios

RORgt suppresses BTLA expression to allow cell activation

Vasileios Bekiaris, John R. Sedy, Carl F. Ware

SanfordlBurnham Medical Research Institute

The TNF superfamily member HVEM (Herpes Virus Entry Mediator) is a highly conserved receptor expressed from jawless fish to humans. HVEM binds the TNF ligands LIGHT and LTa3 as well as the Ig-superfamily receptors BTLA (B and T Lymphocyte Attenuator) and CD160. HVEM and BTLA are co-expressed in lymphoid cells forming a complex in-cis, which inhibits HVEM-induced activation. BTLA is regulated following lymphocyte stimulation, however the mechanism regulating cell intrinsic BTLA expression is unknown. We have identified that the nuclear receptor ROR?t (Retinoic acid related Orphan Receptor gamma-t) acts as a transcriptional repressor of BTLA. Expression of BTLA is inversely proportional to the levels of ROR?t. In lymphocyte populations, innate lymphoid cells with the highest level of ROR?t express the least BTLA. Our results describe a new transcriptional target for ROR?t and reveal a novel regulatory mechanism by which innate lymphoid cells can remain active.

Presenter: Berkley, Amy

CD8+ recent thymic emigrant responses following infection with Listeria monocytogenes containing altered peptide ligands

Amy Berkley, Pamela Fink

Department of Immunology, University of Washington

Recent thymic emigrants (RTEs) are an important, yet understudied, subset of T cells. Using a transgenic (Tg) mouse model in which green fluorescent protein (GFP) is expressed under the RAG2 promoter, we have a non-invasive method for identifying and studying RTEs following their exit from the thymus. Our lab has shown that RTEs are phenotypically and functionally distinct from mature na_ve (MN) T cells. We have previously compared the responses of CD8+ RTEs and MN T cells expressing the high affinity, OT-I Tg T cell receptor that recognizes an ovalbumin (OVA) peptide in the context of H-2Kb. Following infection with Listeria monocytogenes engineered to express OVA (Lm-ova), RTEs form fewer IFN-γ+IL-2+ and KLRG1-CD127+ memory precursor effector cells (MPECs) compared to MN T cells. Despite these differences, RTEs and MN T cells mount a similar effector response following secondary challenge with the same pathogen. To investigate the response of CD8+ RTEs to ligands of varying affinity, we infected mice with Lm-ova containing altered peptide ligands (Lm-APLova) following transfer of sorted RTE and MN CD8+ OT-I TCR Tg T cells. We found that infection with Lm-APLova containing lower affinity ligands induced greater MPEC formation, particularly in MN T cells. Compared to MN T cells, RTEs had higher expression of the adhesion molecules Ly6C and a4 integrin, especially in response to lower affinity ligands. Finally, functional memory and recall responses were seen in both RTEs and MN T cells across all Lm-APLova infections. Together, these data suggest that despite early defects in memory formation, RTEs are ultimately able to generate protective recall responses and that, compared to MN T cells, RTEs appear to be less sensitive to reduced ligand affinity. We hypothesize that RTEs represent a tolerance-prone population and that the ability of TCR Tg RTEs to respond to a wider range of ligand affinities than MN T cells may enhance their susceptibility to tolerance induction to self antigens presented in the absence of signals provided by bacterial infection.

Presenter: Bollyky, Paul

High-molecular weight hyaluronan can substitute for IL-2 in IL-2R signaling

Paul Bollyky, Paul L. Bollyky, Melissa Pickett, Ben A. Falk, Tania Habib, Alice Long, Daniel J. Campbell, Thomas N. Wight, and Gerald T. Nepom

Benaroya research Institute

The IL-2R signaling pathway is critical for immune homeostasis. We have discovered a novel interaction between the IL-2R complex, CD44, and the extracellular matrix (ECM) that suggests tissue integrity cues can substitute for IL-2 in certain settings. We report here that treatment of CD4+ T-cells with high molecular weight hyaluronan (HMW-HA), an ECM component characteristic of healing tissues, promotes rapid STAT5 phosphorylation (pSTAT5) of human CD4+ T-cells. Consistent with this, HMW-HA, can promote human CD4+CD25+ Treg viability and function. Low-molecular weight (LMW-HA), generated from HMW-HA at sites of infection and ongoing inflammation, does not promotes pSTAT5, indicating a decisive requirement for ECM integrity. Antibody mediated crosslinking of the HMW-HA receptor, CD44, similarly promotes pSTAT5 in the absence of TCR ligation or exogenous IL-2. This effect was not inhibited by cyclosporine-A or recombinant IL-2Ra (rIL-2Ra) treatment. Neutralizing Ab directed against either CD122 or CD132 blocked CD44-mediated pSTAT5, indicating dependence on the IL-2R complex. Consistent with this, CD44 was co-immunoprecipitated together with IL-2R complex components. Moreover, CD44-/- mice demonstrated reduced pSTAT5 in response to low-dose IL-2, impaired resolution of inflammation, and diminished numbers of GFP/FoxP3+ cells with increasing age. Together these data suggest a novel role for CD44 and HMW-HA in Treg homeostasis. This is exciting because it indicates that the ECM may impact IL-2R signaling in ways usually ascribed to cytokines.

Presenter: Brempelis, Katherine

Effect of Antigen Release on CD4+ T Cell Activation in the Liver

Katherine Brempelis, Isaac Mohar, I. Nicholas Crispe

University of Washington, Seattle Biomedical Research Institute

Effect of Antigen Release on CD4+ T Cell Activation in the Liver Katherine Brempelis 1,3; Isaac Mohar 3; I. Nicholas Crispe 1,2,3 1-University of Washington, Department of Global Health 2-University of Washington, Department of Immunology 3-Seattle Biomedical Research Institute, Seattle WA The tolerogenic environment of the liver makes it an ideal site for infectious agents such as the hepatitis C virus (HCV), the hepatitis B virus (HBV), and Plasmodium falciparum to establish persistent infection within hepatocytes and evade a robust immune response. Weak CD4+ and CD8+ T cell responses have been associated with chronic HCV infection while strong CD4+ and CD8+ T cell responses have been associated with viral clearance. Past research from our lab shows that transduction of hepatocytes with adeno-associated virus-2 (AAV2) encoding ovalbumin (OVA) resulted in CD8+ but not CD4+ T cell proliferation. We aim to test the hypothesis that sequestration of hepatocellular antigen limits CD4+ T cell priming in the liver. To address this, we have developed a novel AAV8 vector to examine whether release of hepatocellular antigen allows for the generation of a more robust immune response. This vector contains ovalbumin (OVA) and the human heparin-binding EGF-like growth factor (hHB-EGF, diphtheria toxin receptor), so that upon diphtheria toxin administration hepatocyte death will occur, thereby releasing OVA from the hepatocyte. This approach will be systematically modified to ask questions about how release of antigen, in either the presence or lack of certain liver antigen presenting cells and other immune stimuli, affects CD4+ T cell proliferation in the liver.

Presenter: Brunette, Rebecca

The Role of the AIM2-like Receptor Family in DNA Sensing

Rebecca Brunette, Deborah G. Whitley, Daniel B. Stetson

University of Washington

A cytosolic antiviral pathway that detects DNA, termed the IFN-stimulatory DNA (ISD) pathway, has been characterized. In this pathway, cytosolic DNA activates type I IFN in a TBK-1 and IRF-3 dependent manner. However, little is known about the sensors or proximal signaling components of the ISD pathway. Two members of the AIM2-like receptor (ALR) family have been shown to be intracellular DNA sensors. AIM2 activates the inflammasome after recognition of cytosolic DNA while the human family member, IFI16, has been shown to bind DNA and activate IFN-_ in a manner dependent on the endoplasmic reticulum adaptor protein, STING. Both the mouse and human ALR family have additional genes of unknown function. Using IFN-_ reporter assays, immunofluorescence and siRNA knockdowns we are investigating the role of these proteins in DNA sensing.

Presenter: Cambier, CJ

Host and Bacterial Factors Required for Macrophage Migration to Initial Sites of Mycobacterial Infection

CJ Cambier, Lalita Ramakrishnan

University of Washington

The zebrafish - Mycobacterium marinum infection model recapitulates the disease pathology of human tuberculosis and allows visualization and manipulation of the early steps of infection. Infecting bacteria incite the rapid arrival of macrophages by which they are phagocytosed. The infected macrophages traffic to tissues and recruit other uninfected macrophages to form granulomas, the hallmark structures of tuberculosis. The zebrafish has provided insights into the molecular and cellular mechanisms of granuloma formation. However, the mechanisms regulating the first wave of macrophage migration towards extracellular bacteria are not known. We are now able to identify host, as well as pathogen factors that mediate recruitment of macrophages towards bacteria. The host CC chemokine receptor 2 (CCR2) and the mycobacterial phenolic glycolipid are both required to initiate the process of macrophage migration to the infection site. The mechanisms of their actions and their role in infection will be presented.

Presenter: Camfield Lind, Kristin

Tapasin and ERAAP have distinct roles in shaping the pMHC I repertoire

Kristin Camfield Lind, Hernando Escobar, Eduardo Reyes-Vargas, Brant Rudd, Niranjana Nagarajan, Federico Gonzalez, Noriyuki Sato, Julio C. Delgado, Nilabh Shastri and Takayuki Kanaseki

University of California, Berkeley

Stable peptide:MHC I complexes (pMHC I) displayed at the cell surface are key to robust CD8+ T cell immunity. The ER-resident chaparone tapasin and the ER aminopeptidase associated with antigen processing (ERAAP) play important but poorly characterized roles in the peptide selection process. To test how the pMHC I repertoire is altered in the absence of tapasin (Tpn-KO), we analyzed CD8+ T cell responses of B6 (WT) mice immunized with Tpn-KO splenocytes. We found that WT anti-Tpn-KO CD8+ T cell lines made strong responses against Tpn-KO APCs, demonstrating that tapasin-deficiency results in presentation of an altered, immunogenic pMHC I repertoire. However, this novel peptide repertoire does not overlap with the unedited-peptide repertoire found in ERAAP KO cells, indicating that ERAAP and tapasin edit the pMHC I repertoire in distinct ways. Furthermore, analysis of the pMHC I repertoire in Tpn-KO or WT splenocytes by mass spectrometry showed peptides unique to Tpn-KO which differed in their C-termini as well as consensus motifs. Thus, ERAAP and tapasin have independent roles in peptide selection: ERAAP edits the N-terminus of the peptide, whereas tapasin edits the C-terminus.

Presenter: Chognard, Gaelle

Study of IL-23R expressing cells and its biological role in a murine colitis model.

Gaelle Chognard, Bellemare Lisa, Dominguez-Punaro MaryCruz, Lesage Sylvie

HMR Research Center

Recently, Genome Wide Association studies have shown an implication of the interleukin 23 receptor in multiple immune-mediated diseases. This cytokine receptor is composed of two chains (IL-23R and IL-12Rß1) and a mutation in the IL-23R chain confer significant protection against the development of Crohns disease and ulcerative colitis while other variants in this gene confer increased risk. However, little is known about the specific contribution of the IL-23R pathway on disease pathogenesis. Thus, we proposed to study IL-23 receptor in a steady state context by exploiting a murine colitis model. Using IL-23R-GFP reporter mice, we confirm that, under non-inflammatory conditions, IL-23R is mainly expressed by ?dT cells, and by a small proportion by CD4+ T cells. In addition, we find that a considerable fraction of Lti-like cells, whose function is still unknown, express IL-23R. Under inflammatory condition, maintenance of the Th17 cells has been reported to be dependent on IL-23. Surprisingly, we find that in vitro differentiated Th17 cells do not express IL-23R. To further investigate the role of IL-23R in inflammatory conditions, we subject IL-23R-deficient mice to DSS-induced colitis. Although IL-23-deficient mice have been reported to be more susceptible to DSS-induced colitis, we find that both WT and IL-23R-deficient mice exhibit a comparable severity of colitis. Additional studies are clearly required to understand the phenotypic variations between IL23 and IL23R-deficient mice under inflammatory conditions and to unravel the protective role of IL-23R polymorphisms in inflammatory disease susceptibility.

Presenter: Clemons, Corey

IMMUNE PATHWAYS REGULATING THE EXPANSION OF ANTIGEN-SPECIFIC NATURAL REGULATORY T CELLS

Corey Clemons, Shahin Shafiani, Crystal Dinh
University of Washington

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Presenter: Cohen, Heather

ATP hydrolysis regulates classical macrophage activation by a CD39-dependent mechanism

Heather Cohen, David Mosser

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park and The Maryland Pathogen Research Institute

Macrophages play vital roles in directing both the progression and resolution of inflammation. However, the factors present in tissue that induce these disparate macrophage functions remain elusive. At the site of injury, host cells release ATP as a danger signalto warn of tissue damage. High levels of extracellular ATP promote immune cell recruitment and the production of pro-inflammatory cytokines. Excessive ATP levels can lead to severe tissue damage during acute or chronic inflammation. Therefore, it is necessary for the host to regulate ATP levels in the extracellular milieu. This can be achieved by two cellular ecto-enzymes, CD39 and CD73, which convert extracellular ATP into adenosine. We propose that macrophages regulate the expression of these two ecto-enzymes to control the catabolism of ATP to adenosine. Here, we demonstrate that classically activated macrophages exposed to low ATP concentrations develop an anti-inflammatory phenotype with attenuated TNFa and IL-12 production and an increase in IL-10. This modulation of cytokine production is abolished in the presence of adenosine deaminase. Therefore, we hypothesize that the immunosuppressive effects of ATP are due to the rapid conversion of ATP to adenosine by macrophages. Furthermore, by utilizing non-hydrolyzable ATP, a CD39-specific inhibitor, and CD39-deficient macrophages, we show that ATP hydrolysis by macrophages potently diminishes inflammatory cytokine production. Moreover, we investigate the source of ATP in vivo and demonstrate that thrombin-activated platelets release ATP to regulate inflammatory cytokine production from macrophages. We propose that purinergic signaling represents a unique immunoregulatory mechanism that macrophages utilize to control inflammatory disease progression. On-going studies include: 1) assessing the kinetics of ATP hydrolysis and adenosine production by macrophages, 2) investigating the role of extracellular ATP in controlling macrophage function by engineering CD39 and CD73-overexpressing macrophages, and 3) determining the physiological role of ATP hydrolysis in various inflammatory disease models. These studies reveal the remarkable plasticity of macrophages and that these cells can self-regulate their activation state by controlling extracellular ATP levels.

Presenter: Corse, Emily

Chronic autoimmune hepatitis in mice expressing a CD28-specific ligand

Emily Corse, Rachel Gottschalk, P'ng Loke, Timothy Sullivan, Linda Johnson, James Allison
Memorial Sloan-Kettering Cancer Center

Not to be placed on the website.

Presenter: Crampton, Steve P.

Transgenic expression of MDA5 drives a chronic type I IFN gene signature, viral resistance and augments autoimmunity

Steve P. Crampton, Jonathan A. Deane, Lionel Feigenbaum & Silvia Bolland

National Institutes of Health

Type I interferons (IFN-I) are normally produced during antiviral responses, yet high levels of chronic IFN-I expression correlate with autoimmune disease. A variety of viral sensors generate IFN-I in their response, but other than TLRs it is not fully known which pathways are directly involved in the development of spontaneous immune pathologies. To further explore the link between IFN-I induced by viral pathways and autoimmunity, we generated a new transgenic (Tg) mouse line containing multiple copies of Ifih1, a gene encoding the cytoplasmic dsRNA sensor MDA5 with proven linkage to diabetes and lupus. We show that MDA5 overexpression led to a chronic IFN-I state characterized by resistance to a lethal viral infection through rapid clearance of virus in the absence of a CD8+ or antibody response. Spontaneous MDA5 activation was not sufficient to initiate autoimmune or inflammatory pathology by itself even though every immune cell population had signs of interferon activation. When combined with the lupus-susceptible background of the Fc?R2B deficiency, MDA5 overexpression did accelerate the production of switched autoantibodies, the incidence of glomerulonephritis and early lethality. Thus, MDA5 Tg mice provide evidence that chronic elevated levels of IFN-I are not sufficient to initiate autoimmunity or inflammation although they might exacerbate an ongoing autoimmune pathology.

Presenter: Duggan, Jeffrey

DAP12 regulates the innate immune response during Listeria monocytogenes infection.

Jeffrey Duggan, Hammerman, Jessica A.

Dept. of Immunology Univ. of Washington, Benaroya Research Institute at Virginia Mason

Myeloid cells such as macrophages and dendritic cells (DCs) play essential immune functions during the course of infection. Their role in the innate immune response relies on their expression of Pattern Recognition Receptors (PRRs), including the Toll-like receptors (TLRs). While PRR-induced innate responses are vital to host defense against pathogens, they must also be tightly regulated to prevent the development of inflammatory syndromes such as septic shock. DAP12 is an ITAM-containing signaling adaptor expressed in myeloid cells that can inhibit TLR-induced inflammatory responses. To examine the role of DAP12 during systemic infection, we have utilized a model of Listeria monocytogenes infection. Mice deficient in DAP12 have enhanced bacterial clearance compared to WT animals from the spleen and liver at 3 days post-infection. Additionally, DAP12-deficient mice infected with Listeria have higher recruitment of inflammatory monocytes and neutrophils to the spleen at 48 hours post-infection. We hypothesize that the recruitment of these myeloid cell populations results in enhanced bacterial clearance, and that the expansion of inflammatory monocytes leads to the induction of TNF and iNOS-producing (Tip)-DCs required for clearance of systemic Listeria infection. We will further explore the expansion of these myeloid populations and the production of pro-inflammatory cytokines in Listeria-infected DAP12-deficient mice from blood, spleens and livers during the course of infection. We expect these findings to elucidate the role of DAP12 in regulating inflammatory output from myeloid populations during the course of bacterial infection.

Presenter: Eckard, Sterling

Defining Crosstalk Between Stress Responses and Innate Immunity

Sterling Eckard, C.J. Cambier, Daniel B. Stetson

University of Washington Department of Immunology

All cells have sensors that detect nucleic acids and trigger a cell-intrinsic antiviral response through the production of type I interferons. The RNA helicases RIG-I and MDA5 detect viral RNA, whereas some of the sensor(s) for viral DNA remain unknown. DNA exonucleases, particularly 3 Repair Exonuclease 1 (Trex1), regulate the cell-intrinsic antiviral response to DNA ligands. In contrast, nothing is known about exonuclease regulation of RNA-activated antiviral responses. We therefore hypothesize that a system analogous to Trex1 exists for the metabolism of viral RNA ligands. We examined SKIV2L, a component of the 3->5RNA exosome and a recently defined lupus susceptibility allele, as a candidate negative regulator of antiviral RNA sensors. Preliminary evidence suggests that loss of SKIV2L dramatically increases the activation of RIG-I by viral RNA ligands. Remarkably, we find that endogenous RNA cleavage products of the IRE-1 endonuclease generated during the unfolded protein response (UPR) trigger an interferon response in SKIV2L-depleted cells but not in control cells. This finding suggests that SKIV2L prevents the activation of antiviral sensors by endogenous RNA products generated during sterile stress responses. This reveals a molecular mechanism by which cellular stress responses could be misinterpreted as infection, with potential implications for autoimmune disease.

Presenter: Edde, E. Lynn

Human alveolar cells fail to produce PGE2 in vitro after Francisella Infection

E. Lynn Edde, Lydia Barrigan, Shraddha Tuladhar, Jeffrey Frelinger

The University of Arizona, Department of Immunobiology

Problem: In mice we have previously shown that Prostaglandin E2 (PGE2) production is an important mechanism of immune evasion by Francisella. Macrophage cell lines, and murine bone marrow derived macrophages produce PGE2 after infection with Schu S4, live vaccine strain (LVS) and Francisella novicida. Additionally, the blockade of PGE2 production by indomethicin shortens infection and increases IFN-? responses in the lung. In this study, we investigated the ability of human bronchial alveolar lavage (HBAL) macrophages to produce PGE2 after infection in vitro. Surprisingly, HBAL macrophages fail to produce PGE2 following infection. Methods: Human bronchial alveolar lavage is processed to equal 1x106 cells/mL and divided into 96-well plates. The cells are then infected with diminishing MOIs of Francisella novicida and LVS along with 2 similar mutants (?119/dotU). Escherichia coli LPS was used as a control. Twenty-four hours following infection the supernatants were collected and the ELISA was performed for PGE2. Results: The findings show that prostaglandin release occurs following stimulation with Francisella novicida and LPS, but minimal amounts are produced by the mutants (?119/dotU) or LVS. Conclusions: In mice strains LVS and Francisella novicida are highly virulent. The failure of LVS to induce PGE2 in human alveolar cells could explain the diminished virulence of this strain in humans compared to mice. To test this hypothesis, it will be important to examine the ability of the strain SchuS4, which is highly pathogenic in humans, for induction of PGE2 with HBAL.

Presenter: Elsner, Rebecca A

Non-functional CD4+ T helper cell responses to B. burgdorferi

Rebecca A Elsner, Christine J Hastey, Stephen W Barthold, Nicole Baumgarth

Center for Comparative Medicine at UC Davis, Microbiology Graduate Group

Infection with the Lyme disease agent Borrelia burgdorferi (Bb) is characterized by massive accumulation of B cells and antibody secreting plasma cells, and a loss of T cell and B cell area organization in draining lymph nodes. Antibody production is critical for resolution of disease symptoms. However, these antibodies are not functionally altered when generated in the absence of CD4 T cell help. Moreover, infections with Bb do not provide immune protection against reinfection. These observations suggested that Bb-infection might induce defective T-helper cell responses, or that the destruction of the lymph node architecture might inhibit the formation of successful T-dependent B cell responses. To assess T helper cells following Bb-infection we performed multicolor FACS-analysis and histology on draining lymph nodes of mice at various time points after infection with host-adapted Bb. Results showed that CD4 T cells were activated and ICOS+ CXCR5+ TFH formed after the infection, however that the germinal center response was slow and only transiently induced at that site. These data were thus consistent with reduced T-dependent B cell response induction following Bb infection. We next assessed whether the TFH cells were functional. For that we co-cultured TFH from lymph nodes of infected mice with B cells from Bb-immunized mice and measured B cell proliferation. The results revealed a reduced ability of T cells from infected mice to support B cell proliferation, compared to T cells isolated from mice immunized with Bb. Future studies will focus on developing an antigen-specific system to further assess the quality and magnitude of the Bb-specific CD4+ T cell response. To examine the effects of the lost lymph node architecture, we analyzed the ability of the lymph nodes from Bb-infected mice to mount a B cell response after immunization with an unrelated antigen (influenza). Despite the loss of architecture and the lack of germinal center formation at day 10 after Bb-infection, the immunization induced germinal centers, antigen-specific TFH and a robust and specific antibody response. Taken together, the data suggest that a lack of functional TFH cell responses, rather than altered lymph node architecture, impedes the development of functional B cell responses to Bb infection.

Presenter: Epeldegui, Marta

BST2/Tetherin is constitutively expressed in the human the thymus

Marta Epeldegui, Christel UIttenbogaart

University of California Los Angeles

We have previously shown that IFN-alpha is constitutively expressed by plasmacytoid dendritic cells (pDC) in the human thymus and not in the periphery. IFN-alpha secretion by pDC is consistent with an activated phenotype of these cells. Hence, we have expanded our work on thymic pDC by further characterizing their phenotype. Here we show that thymic pDC have a different phenotype than peripheral (PBMC and spleen) pDC. We studied the expression of various cell surface antigens on thymic and peripheral pDC (CD31, BDCA2, BDCA4, BST2, ILT7, CD62L) and observed that thymic pDC express CD31 and BDCA2 at lower levels than peripheral pDC. On the contrary, virtually all (80-90%) thymic pDC express BST2, while only a fraction (50%) of peripheral pDC expresses BST2. In addition, thymic pDC lack the expression of CD62L and ILT7 (BST2 receptor), while the vast majority of peripheral pDC express both CD62L and ILT7. Altogether, the expression or lack of these cell surface markers is consistent with an activated phenotype, which would render pDC the capability to secrete IFN-alpha. Thus, since thymic pDC have an activated phenotype and we have shown that IFN-alpha is constitutively expressed in the thymus at low levels, we investigated whether BST2 (an IFN inducible gene) is expressed on thymocytes in addition to pDC. BST2, also known as Tetherin and CD317, is an antiviral protein that has been known to be naturally expressed on B cells. BST2 has antiviral functions and inhibits HIV by impeding the release of progeny virions from the cell surface. Interestingly, we observed that BST2 in addition to pDC is also expressed constitutively on a subset of thymocytes, in contrast to peripheral T cells which do not express BST2. More concretely, BST2+ thymocytes are mature thymocytes (CD3+CD27+) located in the thymic medulla. These thymocytes also express CD25 and are mainly CD4+, and preferentially express CCR5, which is expressed at low levels in the thymus. To determine whether BST2+ thymocytes are infectable with HIV, but do not release virus due to antiviral function of BST2, we exposed thymocytes to CXCR4 and CCR5 tropic HIV-1 isolates. Our results show that upon HIV infection BST2+ thymocyes have higher levels of HIV-1gag protein intracellularly when compared with BST2-negative thymocytes. This is consistent with the notion that BST2 would be tethering HIV, thereby not allowing it to be released from the cell. We conclude that constitutive expression of BST2 in the human thymus may protect cells in the thymus from the impact of viral replication. However, it is likely that BST2 has also so far unknown functions in T cell development.

Presenter: Ergun, Ayla

The Immunological Genome Project and Differential Splicing Landscape in Immune Cells

Ayla Ergun, James Costello, James Collins, Diane Mathis, Christophe Benoist and the Immunological Genome Project

Harvard Medical School

Not to be placed on the website.

Presenter: Feng, Ting

Genetic Variation of Transcriptional Activation in Human CD4+ T Cells

Ting Feng, Katherine Rothamel, Graeme Doran, Michael Wilson, Yoni Donner, Philip de Jager, Daphne Koller, Diane Mathis, and Christophe Benoist

Department of Microbiology and Immunobiology, Harvard Medical School, 77 Avenue Louis Pasteur, NRB 1052, Boston MA 02115

Not to be placed on the website.

Presenter: Flach, Melanie

Role of Saposin D in cross-presentation of vesicular antigen

Melanie Flach, Florian Winau

Immune Disease Institute, Program in Cellular and Molecular Medicine at Childrens Hospital Boston, Department of Microbiology and Immunobiology at Harvard University, Boston, MA

Not to be placed on the website.

Presenter: Friesen, Travis

Recent thymic emigrants initiate a weak germinal center reaction

Travis Friesen, Deborah W. Hendricks, Pamela J. Fink
University of Washington

Recent thymic emigrants (RTEs) are T cells that have recently undergone thymic development and egress to the lymphoid periphery. RTEs are a clinically relevant population because they play a critical role in establishing the nascent immune system in neonates, as well as reconstituting the na_ve peripheral T cell pool in adults recovering from lymphophenia. To study RTEs, our lab utilizes mice transgenic (Tg) for green fluorescent protein (GFP) driven by the Rag2 promoter. In these RAG2p-GFP Tg mice the GFP signal remains detectable for 2-3 weeks following the loss of Rag2 expression, and therefore tags RTEs in the periphery. Activated CD4+ RTEs have been shown to exhibit a bias toward the Th2 effector lineage, with more robust IL-4, IL-5 and IL-13 production than their mature na_ve (MN) counterparts. We now show that CD4+ RTEs proliferate less than MN T cells in response to antigen in vivo, and have diminished Bcl-6 and elevated Blimp-1 expression. In line with these latter findings and suggesting that RTEs initiate a weaker germinal center reaction, these cells are poor at driving the generation of high affinity Th1-associated antibodies as shown by ELISA. These results further emphasize the phenotypic and functional differences between RTEs and MN T cells, and understanding these differences will be crucial to implementing improvements in neonatal vaccination and developing strategies to accelerate the recovery of the peripheral T cell pool following lymphoablation.

Presenter: FU, WENXIAN

A multiple redundant genetic switch locks in the transcriptional signature of Treg cells

WENXIAN FU, Ayla Ergun, Ting Lu, Jonathan Hill, Sokol Haxhinasto, Marlys Fassett, Roi Gazit, Stanley Adoro, Laurie Glimcher, Susan Chan, Philippe Kastner, Derrick Rossi, James Collins, Diane Mathis, Christophe Benoist

Dept of Microbiology and Immunobiology, Harvard Medical School

Presenter: Fujikado, Noriyuki

Aire must be expressed in perinatal window to guard against autoimmunity

Noriyuki Fujikado, Christophe Benoist, Diane Mathis

Division of Immunology, Harvard Medical School

Aire is a transcriptional regulator that has important functions for establishment of immunological tolerance. Mutations of Aire cause monogenic, multi-organ, autoimmune diseases called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) or autoimmune polyglandular syndrome type-1 (APS-1). Although Aire was known to be expressed in thymic medullary epithelial cells (MECs) from fetal through adult life, at least in mice, whether it impacts on immunological tolerance during this entire time was not known. Using a doxycycline-regulated transgene to target Aire expression to the thymic epithelium, complementing the Aire knockout in a temporally controlled manner, we found that Aire is essential in the perinatal period, but is dispensable in adults, to prevent the multi-organ autoimmunity that is typical of Aire deficiency. One possible explanation for the differential ability of Aire to protect from multi-organ autoimmune disease when expressed in adults versus neonates is that it controls a different repertoire of MEC gene transcripts at the two stages. Therefore, we compared MEC transcripts at the neonatal and adult stages. Microarray-based profiling of total MHC class II-hi MEC showed significant differences in the Aire dependent gene signature, especially for genes that encode peripheral tissue antigens (PTAs), between adults and neonates. The analysis of PTAs showed that transcripts unique to the intestine, liver, or stomach were expressed in adults more than in neonates. Reversely, in neonates, there was a particular pattern of the tissues represented, including transcripts unique to the fetal-life support system such as the uterus, placenta, and umbilical cord, and immunological privileged tissues such as the eye, brain, and gonads. These deta suggest that different PTA repertoires might be expressed during the critical neonatal window. Furthermore, we focused on non-PTA transcripts such as antigen presentation-related genes because it was reported that Aire regulates not only PTA but also non-PTA gene expression. The microarray analysis showed that some antigen-presentation-related genes were differentially expressed in neonates compared with adults. Taken together, Aire must be expressed in the perinatal window to guard against multi-organ autoimmune disease; in the neonatal thymus, a different repertoire of PTAs is expressed and non-PTA genes are also differentially expressed to establish neonatal immunological tolerance.

Presenter: Gebreselassie, Nebiat

Eosinophils and the IL-4/STAT6 axis regulate Trichinella spiralis muscle infection by mediating parasite growth

Nebiat Gebreselassie, Lu Huang, Lucille F. Gagliardo, Nancy A. Lee, James J. Lee, and Judith A. Appleton.
Cornell University

The parasitic nematode Trichinella spiralis establishes chronic infection by invading and developing within skeletal muscle cells. Eosinophils are prominent in cellular infiltrates associated with infected cells. We have shown previously that eosinophils support the growth of T. spiralis larvae and also prevent the development of Th1-driven production of NO that is toxic for growing parasites. Restoring eosinophils to eosinophil-ablated mice improves Th2 cell accumulation at sites of infection, while enhancing parasite growth and survival. Here, we show that STAT6 signaling is a key pathway that regulates parasite growth and is associated with direct effects on glucose metabolism in infected mice. These results begin to define the link between nutrient metabolism and the immune system that occur at a site of chronic nematode infection in which the eosinophil functions as a pivotal regulator of immunity.

Presenter: Graham, Amy C.

Mast cell-dependent inflammatory response during influenza A virus infection

Amy C. Graham, Julie M. Zickovich, Kimberly M. Hilmer, Joshua J. Obar

Montana State University, Department of Immunology & Infectious Diseases, 960 Technology Boulevard, Bozeman MT 59718

The lung is protected from pathogens by alveolar epithelial cells, tissue resident alveolar macrophages, dendritic cells, and mast cells. Pulmonary viral infection can result in severe disease associated with increased vascular permeability. It is well established that mast cells alter vascular permeability under a number of conditions. However, the role of the mast cell has been under explored in these severe pulmonary viral infections. In mice that lack functional mast cells, pathological immune responses are altered depending on the influenza strain. We found that both the A/WSN/33 and A/PR/8/34 influenza viruses generate significant immunopathology in C57BL/6 mice, but only the pathology induced by A/WSN/33 was mast cell dependent. Neutrophil recruitment and inflammatory cytokine production both correlated with increased lung permeability and pathology, as well as systemic disease severity as measured by weight loss. To assess why the inflammatory response to the A/WSN/33, but not the A/PR/8/34, strain of influenza A virus was mast cell dependent, we next examined the ability of each to activate in vitro-derived bone marrow mast cells. We found that that A/WSN/33, but not A/PR/8/34, influenza virus activated bone marrow-derived mast cells to produce inflammatory cytokines/chemokines (IL-6, CCL2-5, TNF-A), anti-viral chemokines (CXCL9 and CXCL10), and degranulate. Importantly, a recombinant A/PR/8/34 virus that expresses the hemagglutinin (HA) glycoprotein from A/WSN/33 instead of its own HA could induce cytokine production from bone marrow-derived mast cells, demonstrating an important role for the HA glycoprotein as a determinant of mast cell activation. Mast cell infection and viral RNA synthesis and export were necessary for mast cell activation. Mast cell activation occurred in a MAVS-dependent mechanism. Thus, we have identified a unique inflammatory cascade which could be targeted to limit morbidity following infection with certain influenza virus strains and potential other pulmonary viral diseases which are associated with increased vascular permeability.

Presenter: Gray, Elizabeth

Identification of a motile IL-17 producing [g]d T cell population in the dermis

Elizabeth Gray, Kazuhiro Suzuki, and Jason Cyster
University of California San Francisco

Dendritic epidermal T cells (DETCs) are a well-studied population of γd T cells that play important roles in wound repair. Here we characterize a second major population of γd T cells in the skin that is present in the dermis. In contrast to DETCs, these Vγ5-negative cells are IL-7Rhi CCR6hi RORγt+ and are pre-committed to IL-17 production. Dermal γd T cells fail to reconstitute following irradiation and bone marrow transplantation unless the mice also receive a transfer of neonatal thymocytes. Real-time intravital imaging of CXCR6-GFP/+ mouse skin reveals dermal γd T cells migrate at ~4Mm/min while DETCs are immobile. Like their counterparts in peripheral lymph nodes, dermal γd T cells rapidly produce IL-17 following exposure to IL-1B plus IL-23. We have characterized a major population of skin γd T cells and propose that these cells are a key source of IL-17 in the early hours after skin infection.

Presenter: Guerau-de-Arellano, Mireia

Immunomodulatory miRNAs in Multiple Sclerosis

Mireia Guerau-de-Arellano, K.M. Smith, J. Godlewski, Y. Liu, R. Winger, S. Lawler, C. Whitacre, A.E. Lovett-Racke and M.K. Racke

The Ohio State University

Not to be placed on the website.

Presenter: Haeussler, Maximilian

Automatically annotating immunoglobulin sequences with the literature

Maximilian Haeussler, Ngan Nguyen, Hyunsung Kim, Martha Zuniga, David Haussler

CBSE, UC Santa Cruz

Thousands of antibody and T-Cell receptor sequences have been described in the immunological literature during the last decades. Some of them have been submitted to databases like Genbank/IMGT but most - especially shorter ones - are still hidden in tables, figures and supplementary files. Modern sequencing machines are able to obtain millions of reads per blood sample, but the experimenter has to trawl manually through the literature to annotate these. Literature and internet search engines hardly make this easier, as they do not allow mismatches or partial matches. We are building the most comprehensive list of previously described immunology-related DNA and protein sequences to date, with direct links to their descriptions. We wrote software that can find molecular sequences in computer files of any format (Internet pages, Microsoft Office documents, PDF, etc). We have run this software on the fulltext of about 3 million research articles, 1 million webpages and for selected sequences searched the whole internet. On the example of TCR high-throughput sequencing datasets from patients suffering from Ankylosing spondilitis, we demonstrate how BLAST-searches against our list of published sequences allow a quicker and more detailed annotation of CDR3 sequences than previously possible.

Presenter: Hamerman, Jessica A.

Type I IFN dependent alterations in myelopoiesis in mice overexpressing TLR7

Jessica A. Hamerman, Matthew B. Buechler, Xizhang Sun, Tom Teal, Keith B. Elkon

Benaroya Research Institute

Mice overexpressing Toll-like Receptor 7 (TLR7.1 mice) have been previously described as having splenomegaly associated with an expansion of CD11c+CD11b+ cells. Here, we examined these mice more closely and observed that the percentage of splenic inflammatory monocytes and neutrophils was increased, whereas the percentage of splenic dendritic cell subsets was decreased, compared to wild-type mice (WT). Investigation of lineage negative hematopoietic precursors in the bone marrow revealed that TLR7.1 mice had increased numbers of primitive precursor cells and granulocyte macrophage progenitors, but not common myeloid progenitors. Analysis of mixed bone marrow chimeras and TLR7.1 mice lacking the IFNa receptor showed that this phenotype was largely cell-extrinsic and partially dependent on Type I IFN signaling. Plasmacytoid DC in the bone marrow and spleen from TLR7.1 mice upregulated MHCII in a cell-intrinsic manner and pDCs, but not other cells, generated mRNA for Ifnb1. This study supports a model in which TLR7 overexpression drives the activation of plasmacytoid dendritic cells, which secrete Type I IFN that acts on hematopoietic progenitor cells to influence myelopoiesis.

Presenter: Han, Hongwei

Thymic stromal lymphopoietin (TSLP)-mediated dermal inflammation aggravates experimental asthma

Hongwei Han, Whitney Xu, Mark B. Headley, Michael R. Comeau, Steven F. Ziegler

Benaroya Research Institute

Individuals with one atopic disease are far more likely to develop a second. For example, approximately half of all atopic dermatitis (AD) patients subsequently develop asthma, particularly those with severe AD. This association, suggesting a role for AD as an entry point for subsequent allergic disease, is a phenomenon known as the atopic march. While the underlying cause of the atopic march remains unknown, recent evidence suggests a role for the cytokine TSLP. We have established a mouse model to determine whether TSLP plays a role in this phenomenon, and in this study show that mice exposed to the antigen OVA in the skin in the presence of TSLP develop severe airway inflammation when later challenged with the same antigen in the lung. Interestingly, neither TSLP production in the lung nor circulating TSLP is required to aggravate the asthma that was induced upon subsequent antigen challenge. However, CD4 T cells are required in the challenge phase of the response, as was challenge with the sensitizing antigen, demonstrating that the response was antigen-specific. This study, which provides a clean mouse model to study human atopic march, indicates that skin-derived TSLP may represent an important factor that triggers progression from atopic dermatitis to asthma.

Presenter: Han-Kyul , Kim

Cisplatin induced cell cycle arrest through CDKN1A, CDKN2B and CDKN2

Kim Han-Kyul , Hyuk-Kwon Kwon, Yong-Min Choi, Sangdun Choi

Ajou university

Not to be placed on the website.

Presenter: HASHIMOTO, Kahoko

The role of Sec8 molecule dependent and independent antigen uptake mechanism in antigen presenting cells.

Kahoko HASHIMOTO, Shu MATSUNAGA, Ken TAKAHASHI

Chiba Institute of Technologgy

Many candidate genes have been discussed for the susceptibility to Rheumatoid arthritis (RA). Among those genes, we have focused on the function of SEC8L1 gene to study for both cell-mediated and humoral immune reaction. It is indicated that the gene may contribute to development of synovium, also the inflammation is involved in type I helper T cells, activated B cells, dendritic cells (DC), macrophages, as well as other inflammatory cells. SEC8L1 gene encodes the Sec8 protein, a component of the Exocyst complex. The Exocyst complex is known for assembly organelle when intracellular vesicle is fused to the target membrane. To study the function of Sec8 molecules in antigen presenting cells, by using macrophage cell line RAW264.7, we investigated Sec8 localization and organelle localization with several types of antigens, such as dextran, OVA, microbead, and also by using several size of antigens. When small size antigens were incorporated via endocytosis, Sec8 were indicated near the antigen area, from early endosome to lysosome area, although when relatively large antigens were incorporated by endocytosis, Sec8 did not show the co-localization with antigens or such organelle. With RNA interference system, SEC8L1 gene expression was knocked-down, the loss of antigen uptake effect was measured as well as the efficacy of antigen presentation.

Presenter: Hastey, Christine

Delays and diversions mark the development of B cell responses to Borrelia burgdorferi infection

Christine Hastey, Rebecca Elsner, Steve Barthold, Nicole Baumgarth

Center for Comparative Medicine, UCD

B cells control disease symptoms of infection with Borelia burgdorferi, a spirochete that causes chronic, non-resolving Lyme disease, but are unable to clear the infection and to prevent reinfection. Previous studies suggested the induction of mainly T-independent B cell responses, potentially explaining some of these findings. However, others showed clear effects of T cells on the magnitude of the B. burgdorferi-specific B cell response. This study aimed to provide a clearer picture of the humoral response to B. burgdorferi and its degree of T cell-dependence, with the ultimate goal of elucidating the mechanisms underlying the failure of successful immunity to this emerging infectious disease. Our study demonstrates the presence of distinct stages in the B cell response, all dominated by the generation of T-dependent and T-independent antibodies of the IgM isotype: A first wave of strong T-independent B cell accumulation in lymph nodes and the induction of Bb-specific antibody responses in the virtual absence of germinal centers. A second wave beginning not until week 2 _ - 3, in which relatively short-lived germinal centers develop in lymph nodes, despite a lymph node architecture that lacks clearly demarcated T and B cell zones. This response failed, however, to generate appreciable numbers of long-lived bone marrow plasma cells. Finally, the accumulation of long-lived antibody-secreting plasma cells >3 months after infection, reflected in a strong, but ultimately non-functional serum antibody response. Overall, the study indicates that B. burgdorferi might evade B cell immunity by interfering with the response kinetics and its quality.

Presenter: Heeke, Darren

Development of guinea pig in vivo model for vaccine/adjuvant testing

Darren Heeke, Eileen Rao, Jason Marshall, Jennifer Woo

MedImmune Vaccines

Although mice have been dominant as the in vivo model of choice for initial nonclinical vaccine evaluations, wider use of guinea pigs (GPs) has emerged because of their susceptibility to infection by viruses such as HSV and FMDV, and their similarity to human responses for asthma and viral respiratory disease compared to mouse models. In order to utilize the GP as a model for adjuvant development, we have developed several assays to measure humoral and cell-mediated immune responses in GPs to TLR (Toll-like receptor) agonist-based adjuvant compounds. To determine which TLRL (TLR ligand)-based adjuvant formulations would be active in GPs, we developed in vitro screening assays to measure GP cytokine responses to TLRLs (Toll-like receptor ligands). A human IL-8 ELISA kit was utilized to measure secreted, cross-reactive GP IL-8, while the Affymetrix QuantiGene assay measured GP cytokine gene expression by in vitro stimulated PBMCs. This information led to the selection of the TLRLs MPL (TLR4), CpG-2395 (TLR9), Gardiquimod (TLR7) and poly(I:C) (TLR3) to be used with an oil-in-water emulsion as a delivery vehicle to adjuvant viral subunit antigens in GPs. Surrogate antigens used to facilitate adjuvant evaluation included antigens derived from common human pathogens like RSV and CMV. Antibody responses were analyzed by IgG ELISA and neutralizing Ab assays. CMI responses of PBMCs from immunized animals were characterized by IFN-g ELISPOT, QuantiGene analysis of cytokine profile, and CFSE-FACS analysis of antigen-specific CD4 T cell proliferation. In addition, CD8 CMI responses were identified by ELISPOT analysis of PBMCs depleted of CD4 T cells via MACS. These assays have served to identify preparations that incorporate TLR4Ls and/or TLR9Ls delivered in oil-in-water emulsion as robust adjuvants for vaccination of GPs.

Presenter: Higdon, Lauren

The role of germinal centers in T cell receptor revision

Lauren Higdon, Pamela Fink

University of Washington Department of Immunology

CD4+ T cells in VB5 transgenic mice become tolerant to an endogenous superantigen (Mtv-8) through either deletion or T cell receptor (TCR) revision. In the revision process, T cells downregulate surface VB5 expression and undergo RAG-mediated rearrangement and expression of endogenous TCRs. Given that revision occurs in germinal centers (GCs), and GC T cells in VB5 Tg mice increase in an Mtv-8-dependent manner, we are studying whether revising cells have a follicular helper T cell (Tfh) phenotype and whether GC interactions are required for revision. Revising cells have an RNA and surface phenotype resembling that of Tfh and distinct from that of post-revision T cells, with elevated Bcl-6, CXCR5, and PD-1, and low Blimp-1 expression. The adaptor molecule SAP is required for prolonged interactions of B and T cells in the GC, and SAP null mice have reduced numbers of post-revision T cells, but no reduction in revision intermediates. These results suggest that revising T cells share some traits with Tfh and that prolonged B-T cell interactions are required for completion, but not initiation, of TCR revision.

Presenter: Hyuk-Kwon, kwon

Cisplatin induced nephrotoxicity via the ATF3

kwon Hyuk-Kwon, Yong-Min Choi, Sangdun Choi
Ajou university

Not to be placed on the website.

Presenter: Ji, Yun

Repression of the DNA-binding inhibitor Id3 by Blimp-1 limits CD8+ T cell memory formation

Yun Ji, Zoltan Pos, Mahadev Rao, Christopher A. Klebanoff, Zhiya Yu, Madhusudhanan Sukumar, Robert N. Reger, Douglas C. Palmer, Zachary A. Borman, Pawel Muranski, Ena Wang, David S. Schrump, Francesco M. Marincola, Nicholas P. Restifo, Luca Gattinoni

NIH/NCI

Blimp-1 is a transcriptional repressor that promotes the differentiation of CD8+ T cells into shortlived Klrg1+ effector cells (SLEC), but how it operates remains poorly defined. Here we show that Blimp-1 and Id3 are reciprocally expressed at both mRNA and protein levels. Blimp-1 binds the Id3 promoter and represses its expression in effector CD8+ T cells similar to what is observed in B cells. Id3-deficient CD8+ T cells had severely impaired long-term survival indicating that Id3 is essential for CD8+ memory T cell formation and downregulation of Id3 by Blimp-1 is a programmed switch that controls the survival of effector T cell populations and their maturation into longlived memory T cells. Furthermore, Enforced expression of Id3 was sufficient to rescue SLEC survival and Id3 overexpressing memory T cells displayed enhanced recall responses and prolonged host survival upon tumor challenge. We performed transcriptome comparison of Id3 loss and gain-of-function cells and observed strikingly similar transcriptional differences between Id3/ versus Id3+/+ and WT versus Id3-overexpressing T cells. A coordinated expression of genes involved in DNA-replication and repair in these two gene expression profiles suggests that Id3 promotes survival of CD8+ effector T cells through inducing genes regulating genome stability. E2a was identified as a downstream factor inhibited by Id3 using Gene Set Enrichment Analysis. Their interaction was further supported by Electrophoretic Mobility Shift Assay result showing that the binding of E2a with its targeted DNA sequence was attenuated in Id3-overexpressing cells. Consistent with the view that E2a and Id3 acted reciprocally, E2a-deficient cells had enhanced longterm survival. These findings identify a Blimp-1Id3E2a axis as a key molecular switch that determines whether effector CD8+ T cells are programmed to die or enter the memory pool.

Presenter: Kageyama, Robin

Ly108 functions as a SAP-dependent on-off switch for T cell help to B cells and NKT development

Robin Kageyama, Fang Zhao, Jennifer L. Cannons, Isharat Yusuf, Christopher Lao, Pamela L. Schwartzberg, Shane Crotty

La Jolla Institute for Allergy and Immunology, and UCSF

Not to be placed on the website.

Presenter: Karnell, Jodi

Mycophenolic acid targets primary B cells but does not inhibit terminally differentiated plasma cells

Jodi Karnell, Fredrick G. Karnell III., Geoffrey L. Stephens, Bhargavi Rajan, Chris Morehouse, Ying Li, Bonnie Swerdlow, Mildred Wilson, Raphaela Goldbach-Mansky, Christopher Groves, Anthony J. Coyle, Ronald Herbst, Rachel Ettinger

MedImmune

Mycophenolic acid (MPA) is an immunosuppressant agent used clinically to prevent transplant rejection and to treat various autoimmune diseases. The production of antibodies directed against self proteins is characteristic of many autoimmune disorders and can contribute to disease pathogenesis. Agents, therefore, which can target antibody production from newly activated B cells as well as long-lived plasma cells, will likely provide therapeutic benefit for patients with autoimmunity. The goal of these studies was to elucidate the mechanisms of action of MPA on B cells isolated from both healthy individuals and autoimmune patients. We show that MPA significantly inhibited both proliferation and differentiation of primary human B cells stimulated under various conditions. Importantly, MPA did not globally suppress B cell responsiveness or simply induce cell death, but rather selectively inhibited early activation events and arrested cells in the G0/G1 phase of the cell cycle. MPA also selectively blocked specific cytokines from activated T cells; surprisingly, while it did not alter IL-2, IL-4 or IL-17, it did inhibit IL-5, IFN-γ and IL-21 production. Furthermore, MPA blocked expansion of both na_ve and memory B cells, as well as prevented plasma cell (PC) differentiation and antibody production from healthy controls and individuals with rheumatoid arthritis. Finally, while MPA potently suppressed Ig secretion from activated primary B cells, terminally differentiated PCs were not susceptible to inhibition by MPA. The target of MPA, IMPDH2, was found to be down-regulated in PCs, likely explaining the resistance of these cells to MPA. These results suggest that MPA provides benefit in settings of autoimmunity by directly preventing activation and PC differentiation of B cells; however, MPA is unlikely to impact autoantibody production by pre-existing, long-lived PCs. These results have implications in therapeutic settings where long lived plasma cells contribute to autoantibody production and disease severity.

Presenter: Kim, Hyunsung

HLA Typing and IG Repertoire Extraction from RNA-Seq

Hyunsung Kim, Zuniga M, Pourmand N
UC Santa Cruz

The emergence of high throughput RNA sequencing (RNA-seq) has caused a fundamental shift in biological expression studies by enabling researchers to examine an entire transcriptome for biologically relevant information. These analyses often rely on bioinformatics tools that assume low levels of mutation in the genome in order to quickly analyze the large amounts of data produced in each experiment. Thus, immunologically relevant information is often discarded due to a lack of genomic conservation in the MHC and regions coding for adaptive immunity. This is in part due to the unusually high mutation rate in the MHC and generation of random genomic sequence during VDJ recombination. Here, a tool that extracts HLA haplotypes and adaptive immune repertoire information from RNA-seq data is presented. HLA haplotypes are called by filtering reads, generating unphased de novo assemblies of each HLA molecule, which are then phased by maximum likelihood estimation. Extraction of adaptive immune repertoire information is performed by filtering reads that map to Immunoglobulin or T-cell Receptor gene segments, identifying complementarity determining regions, and translating clonotypes into amino acid sequences. The frequencies of Variable, Diversity or Joining gene segments and intensities of clonal expansion are visualized to follow changes in the immune repertoire.

Presenter: Korn, Lisa L.

MHC II independent regulation of Tregs by microbial gut flora

Lisa L. Korn, Harper Hubbeling, Terri Laufer
University of Pennsylvania

Not to be placed on the website.

Presenter: Kuriakose, Teneema

Tumor progression locus2 regulates virus replication and antiviral signaling through pattern recognition receptors

Teneema Kuriakose, Wendy T. Watford

The University of Georgia

Not to be placed on the website.

Presenter: Labrecque, Nathalie

A NON-REDUNDANT ROLE FOR NEURON-ORPHAN RECEPTOR 1 (NOR-1) IN THYMOCYTE DEVELOPMENT

Nathalie Labrecque, Salix Boulet, Jean-Fran_ois Daudelin

Maisonneuve-Rosemont Hospital Research Center, University of Montreal

Immature thymocytes expressing auto-reactive T cell receptors (TCRs) are eliminated from the T cell pool via negative selection. This thymic checkpoint results in the deletion of cells that express a TCR of high affinity for self-peptide:major histocompatibility complex (MHC) molecules. In such cases, a strong intracellular signal is transmitted upon TCR:peptide:MHC interaction, leading to apoptosis. Only thymocytes surviving negative selection will mature to the single positive (SP) CD4+ or CD8+ stage. The orphan nuclear receptors Nur77 and Nor-1 are homologous transcription factors (TFs) induced upon TCR signaling. In addition to their role as TFs, they favor negative selection through the conversion of Bcl-2 into a pro-apoptotic factor. It has been suggested that redundancy of Nor-1 and Nur77 could account for the lack of phenotype observed in Nur77-deficient mice. However, the thymus of Nor-1 deficient mice (-/-) has not yet been studied. We have characterized the thymocyte differentiation in Nor-1-/- mice by flow cytometry and identified a distinct population of SPCD8+ T cells. These thymocytes accumulate as mice age and express markers indicative of T cell activation (CD44, CD69, PD-1, CD25). This population also has decreased expression of TCR, CD3, CD24 and CD62L when compared to wild-type SPCD8+ thymocytes. In mice deficient for Rlk and Itk, which are implicated in negative selection, a similar phenotype termed innate memory has been described. However, in Nor-1-/- mice, as opposed to Itk or Rlk deficient mice, IL-4 stimulation does not induce CD124 upregulation on SPCD8+CD44+ cells and the frequency of CD1d+ cells is not increased. This indicates that Nor-1 deficiency does not induce innate memory differentiation. Our data is rather concordant with the hypothesis that the activated SPCD8+ found in Nor-1-/- mice survived negative selection despite having received a strong TCR signal. In support of this, activated SPCD8+ thymocytes accumulate in the thymus of Nor-1-/- mice after anti-CD3 injection. In conclusion, despite the presumed redundancy between Nur77 and Nor-1 in negative selection, the thymic phenotype of Nor-1-/- mice suggests a unique role for Nor-1 in T cell differentiation. Funded by the Canadian Institutes of Health Research

Presenter: Lane, Julie

Current HLA Genotyping Methods: Cost vs. Resolution

Julie Lane, Jacquelyn Knapp, Janelle A. Noble

Children's Hospital and Research Institute Oakland

The major histocompatibility complex (MHC) is the most polymorphic region in the human genome. The HLA Class I and Class II gene families within the MHC have been extensively studied, and multiple alleles are strongly associated with autoimmune diseases, infectious diseases, and adverse drug interactions. HLA genotyping is a necessary component of gene-association studies for diseases with a known or suspected HLA risk factor. In clinical settings, HLA genotypes are the critical determining factor when matching donors to transplant recipients. HLA genotyping is also used to identify patients at risk for certain adverse drug interactions. Historically, HLA genotyping has been both time consuming and expensive, with a trade-off between resources used and level of resolution obtained. Here we present a brief comparison of cost vs. resolution for three HLA genotyping methods.

Presenter: Lau, Laura

Innate Immune Detection of Oncogenic Transformation

Laura Lau, Dan Stetson
University of Washington

A key aspect of antiviral immunity is the induction of type I interferons (IFN) to mediate the effective clearance of a viral infection. The interferon stimulatory DNA (ISD) pathway detects cytosolic DNA in mammalian cells and initiates the activation of a robust antiviral IFN response. Downstream of this pathway is an ER resident adapter protein, stimulator of IFN genes (STING), which is essential for ISD signaling. Interestingly, the ISD pathway is highly active in primary cells but is completely absent from all transformed and tumor cell lines we have tested. This leads us to ask whether the ISD pathway can detect genome instability/oncogenic transformation, and whether there is a selective pressure to lose the ISD pathway in transformed cells. To test this, we are tracking ISD signaling in fibroblasts transduced with defined cellular and viral oncogenes to establish when this pathway is lost during transformation. Moreover, we are comparing the growth rates and onset of senescence between wild-type and STING-deficient fibroblasts during immortalization and transformation. Preliminary evidence suggests that STING-dependent signaling enforces oncogene-induced senescence (OIS) in primary fibroblasts.

Presenter: Lee, Hyeon Woo

Transmembrane protein 126A (TMEM126A) is involved in CD137L-mediated reverse signals in myeloid cells

Hyeon Woo Lee, *Jun Sang Bae*
Kyung Hee University

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Presenter: Li, Bin

PIM1 kinase negatively regulates FOXP3-mediated Transcriptional Regulation

Bin Li, Zhiyuan Li, Guoping Deng, Zuojia Chen, Fang Lin, Yangyang Li, Andy Tsun, Mark I Greene

Institut Pasteur of Shanghai, Chinese Academy of Sciences

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Presenter: Lilley, Graham

SLAMf6 inhibits NKT cell proliferation

Graham Lilley, Jonathan Boyson
University of Vermont

NKT cells are an unusual subset of innate-like CD1d-restricted T lymphocytes that, upon activation, rapidly secrete both pro- and anti-inflammatory cytokines. As such, these cells exhibit protective functions including tumor surveillance and defense against bacteria, viruses, fungi and protozoan parasites, as well as deleterious functions such as increased severity of autoimmune disease and asthma. NKT cell number and overall function vary widely in both the mouse and human populations. This natural variability is thought to contribute both to disease resistance and susceptibility, though the mechanism responsible for this variability is not understood. In mice, genetic control of NKT cell number and function has been linked to a region on chromosome 1 encoding the Slam family of immunoreceptors, which comprises nine cell surface receptors that engage in homotypic binding. Slam family receptors have been implicated in the development of NKT cells, as well as the function of both innate and adaptive leukocytes including NK cells, neutrophils, B cells and T cells. Using co-cultures of CD1d tetramer-reactive NKT cells and aGalCer-loaded murine fibroblasts (L929) stably expressing CD1d and SLAMf6 we show that cross-linking SLAMf6 causes a drastic inhibition of NKT cell proliferation, as measured by CFSE staining, as well as a modest increase in NKT cell apoptosis, as measured by TUNEL staining. Consistent with these findings, we see reduced IL-2 production from NKT hybridomas that stably express SLAMf6, when they are cultured on plates coated with a-CD3 and a-SLAMf6 antibodies, or co-cultured with aGalCer-loaded L929 cells expressing CD1d and SLAMf6. These findings could help elucidate how Slam family receptors exert their control over NKT cells in the periphery and shed light into the mechanism controlling the genetic regulation of NKT cell homeostasis.

Presenter: Manirarora, Jean Nepomuscene

Naturally occurring regulatory T cells expanded in vivo prevent type-1 diabetes in NOD mice

Jean Nepomuscene Manirarora, Linyi Zhang, Cheng-Hong Wei

Center for Biologics Evaluation and Research, Division of Cellular and Gene Therapies, US Food and Drug Administration, Bethesda, MD 20892

Type-1 diabetes is an autoimmune disease caused by the destruction of insulin-producing pancreatic beta cells by auto-reactive T cells. It results from a failure of the immune system to maintain tolerance to self-antigens. Naturally occurring regulatory T cells represent one major mechanism by which auto-reactive T cells are controlled in the periphery. Deficiency in this cell population number or function could tilt the balance between regulatory cells and pathogenic cells and lead to type-1 diabetes development. In this study, we describe a new method to expand antigen-specific regulatory T cells from autoimmune-prone non-obese diabetic (NOD) mice. Naturally occurring regulatory T cells were expanded in vivo using a combination of IL-2, anti-IL-2 antibody, BDC 2.5 peptide, and rapamycin. The expanded cells expressed a classical phenotype of naturally occurring regulatory T cells, displayed suppressive functions in vivo and in vitro and delayed diabetes development in NOD mice. This new method of peripheral tolerance induction could be used in humans to promote tolerance to islet cell transplants without relying on immunosuppressive drugs. Supported by ORISE fellowship and a grant from US Food and Drug Administration, Center for Biologics Evaluation and Research

Presenter: Maschmeyer, Patrick

Liver instructs T cell tropism for the gut by retinoic acid producing hepatic stellate cells

Patrick Maschmeyer1, Ingo Klein2, Florian Winau1

1Immune Disease Institute, Program in Cellular and Molecular Medicine at Childrens Hospital, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, USA. 2Department of General Surgery, University of Wuerzburg Hospital, Wuerzburg, Germany.

Not to be placed on the website.

Presenter: Mathieu, M_lissa

CD40-ACTIVATED B CELLS VACCINATION LEADS TO EFFICIENT GENERATION OF FUNCTIONNAL CD8+ EFFECTORS

M_lissa Mathieu, Jean-Fran_ois Daudelin, Natacha Cotta-Grand, Salix Boulet, R_jean Lapointe and Nathalie Labrecque

Maisonneuve-Rosemont Hospital Research Center, University of Montreal

New ways to restore the immune responses are needed to fight some diseases like cancer. Many dendritic cell (DC)-based vaccines have been tested against established tumor with limited success. One of the difficulties with DC vaccination is to generate enough cells. One solution could be to use activated B cells as APCs to prime cytotoxic CD8+ T cells against tumor antigens (Ag) since B cells can be easily isolated from patient blood and expanded in very high numbers. Our aim was to characterize the CD8+ T cell response induced by activated B cells presenting Ag. To do so, mouse splenocytes were cultured on fibroblasts expressing CD40L to activate and expand B cells (CD40-B). These B cells were matured with LPS, loaded with an ovalbumin peptide (OVA) and used to immunize mice transferred with OVA specific T cells. Our results show that CD40-B LPS express high levels of CD80 and CD86. Immunization with these CD40-B LPS cells generated an effector response that reached numbers almost as high as with DCs immunization. All effectors had an activated phenotype (CD44hi, 1B11hi, CD127lo) and produced IFN-g and IL-2. In vivo killing assays demonstrated that effectors kill specific target cells. Furthermore, these effectors were able to control a Listeria monocytogenes infection. However, B cell immunization did not lead to efficient generation of CD8+ memory T cells while they were generated after DC immunization. We noticed that CD40-B produced less IL-6 and IL-12 compared to DCs but adding these cytokines at the time of immunization did not restore memory formation. Furthermore, a microarray analysis of the effectors showed that many genes were differently expressed following DC or CD40-B cell immunization suggesting that different signals were given by these APCs. We are still actively investigating why B cell vaccination is defective in generating memory T cells. We now possess a nice model to study which signals should be given by APCs for efficient development of memory T cells. Successful B cell immunization may lead to new vaccine strategy to fight cancer and infection. Funded by the Canadian Institutes of Health Research

Presenter: Minhas, Ujla

Role of reactive oxygen species in pathogenesis of pristane induced autoimmunity in female Balb/c model of SLE-like disease.

Ujla Minhas, Minhas Ujla, Das Prabir, Bhatnagar Archana

Department of Biochemistry, Panjab University

Pristane induced lupus in mice indicate an environmentally induced lupus which is widely explored for unravelling the mystery of pathogenesis of disease. Intraperitoneal innate immune reaction to pristane is mainly responsible for the development of SLE-like disease in the model. Reactive oxygen species (ROS) levels were measured in peritoneum of Balb/c model of SLE-like disease after six months of single intraperitoneal injection of pristane. Reactive intermediates (ROS) generation by peritoneal macrophages were significantly enhanced (mean fluorescence value _ SD: 648 _ 100.9) in pristane treated mice (PT) as compared to control mice (mean fluorescence value _ SD: 79 _ 7.8) treated with phosphate buffer saline (PBST). Immunofluorescence study reveals localization of ROS with in the nuclei suggesting oxidative damage. This finding lead to speculations about the role of ROS in the development of lupus. Therefore, this study proposes that the sustained production of reactive intermediates during chronic intraperitoneal inflammation might decline the antioxidant defence and lead to oxidative stress condition which might further be responsible for the autoimmune condition.

Presenter: Moguche, Albanus

A self-renewing CD4+ T cell population is required to maintain long-lived antigen-specific Th1 T responses during chronic Mycobacterium tuberculosis infection

Albanus Moguche, Shahin Shafiani, Crystal Dihn, CJ Cambier, Kevin Urdahl

University of Washington

Mycobacterium tuberculosis (Mtb) is one of the most successful pathogens known today with more than 2 billion people infected worldwide, 90-95% of who are latently infected. CD4+ effector T cells are critical for the control of Mtb infection and in the mouse model, antigen-specific, IFN-γ-producing Th1 cells persist for over a year even in thymectomized animals. This differs from other chronic infections where clonal populations of antigen specific T cells undergo functional exhaustion and eventual death in the event of persistent antigenic stimulation. In the case of Mtb, how these antigen specific cells are maintained despite chronic antigenic stimulation during persistent Mtb infection is not understood. Using MHC class II restricted tetramers, we have recently shown that Mtb-specific CD4 T cells can be grouped into two groups: 1) KLRG1+ cells that proliferate poorly but produce IFN-γ, and 2) PD-1+ cells that proliferate robustly but produce less IFN-γ. Interestingly, transfer experiments showed that the PD-1+ population can differentiate into KLRG1+ but not vice versa. Initial characterization of the PD1+ cells showed that they are a heterogeneous population of cells with different phenotypes. Among these are PD1hiCXCR5hi cells that are also ICOS+ and express Bcl6. These cells closely resemble TFH cells and significantly, they appear in the lungs about 6 weeks post infection when germinal center like structures begin to form within the granulomas. The other subset composed of PD1+ CXCR5 intermediate cells closely resembles T central memory T cells. The final subset of the PD1+ cells is composed of ICOS+ but CXCR5- cells. Here we hypothesize that within the PD1+ population of Mtb-specific CD4 T cells there is a subset of cells serve as the engineof the Th1 response during chronic tuberculosis by undergoing robust proliferation and having the capacity to differentiate into effector Th1 cells that proliferate less, have shorter lifespan, but produce higher amounts of IFN-γ. We are currently investigating the role of B cells, CXCR5, ICOS, and bcl-6 in the maintenance of the antigen-specific CD4 T cell response during chronic Mtb infection.

Presenter: Mohar, Isaac

Kupffer cells exhibit tissue repair phenotype in response to CD8+ T cell-mediated liver immunopathology

Isaac Mohar, Katherine Brempelis, Nick Crispe

Seattle Biomedical Research Institute

Macrophage are generally regarded as pro-inflammatory cells that are observed in regions of active immune response. Kupffer cells are abundant resident macrophage in the liver, residing in the blood space of the narrow sinusoids. Here, we investigated the response of Kupffer cells over a time-course of acute hepatitis induced by CD8+ T cells responding to antigen-expressing hepatocytes. Replication-deficient recombinant Adeno-associated virus serotype-8 was used to transduce 5-10% of hepatocytes to express mCherry-SIINFEKL. Three weeks later, we initiated acute immunopathology by adoptive transfer of 1x10^6 na_ve OTI (SIINFEKL-specific) CD8+ T cells (OTI). Mice were collected on days 2, 4, 6, 8, 10, and 15 after OTI transfer. Serum alanine aminotransferase activity showed peak liver damage at day 8 (ALT > 500 U/L), which correlated with the number of OTIs in the liver. By day 15, ALT levels returned to near normal, fewer OTIs were observed, and mCherry-SIINFEKL expression (by quantitative real-time PCR(qRT-PCR) and fluorescent microscopy) was suppressed or absent. Liver sections stained for F4/80, a highly expressed glycoprotein on mature macrophage, illustrated macrophage-rich lesions containing apoptotic hepatocytes and OTIs and that correlated with damage. Transcriptional analysis of whole liver by qRT-PCR showed hallmarks of inflammation (IFNγ, TNFA) and tissue repair (Collagen1a1, Col3a1, Timp1) with modest increases (2 4-fold) in co-stimulatory molecules (CD80, CD86, CD40, Icam1). These changes in gene expression correlated with tissue damage and OTI abundance, and disappeared by day 15, as the hepatitis and CD8+ T cell response resolved. From these same mice, Kupffer cells (Tie-2(lo), CD11b(hi), F4/80(hi)) were isolated using FACS. Transcriptional analysis of Kupffer cells under resting conditions showed high level expression of co-stimulatory molecules and cytokines, including TNFA, IL-6, and IL-10, consistent with their reputation as active immune cells. To our surprise, however, under active acute immunopathology Kupffer cells drastically down-regulated these cytokines and strikingly up-regulated the expression of genes related to tissue repair, as well as iNOS, which synthesizes the potent vasodilator nitric oxide. These data suggest that the macrophage-rich lesions are regions of active immunopathology and tissue repair, and place the Kupffer cells and recruited macrophage as important cells in wound healing during inflammatory immunopathology in the liver.

Presenter: Murray, Sara

The role of CD4+ T cell help in priming anti-malaria CD8+ memory T cells

Sara Murray, Sebastian A Mikolajczak, Isaac Mohar, Jessica Spahn, Ashley M Vaughan, Stefan H I Kappe, I Nicholas Crispe

Seattle Biomedical Research Institute, University of Washington Pathobiology

Little is known about the immune response required for protection against liver stage malaria infection. CD8+ T cells are key mediators of protective immunity, but it is unclear how parasite antigen is presented to prime these cells and whether CD4+ T cell help is required to program long lasting memory T cells. The lack of protective T cell responses to natural malaria infection may be due to ineffective T cell priming in the liver. The wild-type Plasmodium parasite may block the death of infected hepatocytes, effectively sequestering antigen inside cells that cannot present antigen on MHC class II to prime CD4+ T cells. Genetically attenuated parasite (GAP) vaccine strains induce sterilizing immunity against liver stage infection in mice. GAP priming of protective T cells likely involves the release of parasite antigen from infected hepatocytes to antigen presenting cells, which can then prime CD8+ T cells with CD4+ T cell help. Preliminary data suggests that mice deficient in MHC class II or CD40 are not protected against wild-type infection by GAP immunization. This may indicate that CD4+ T cell help plays a critical role in protective immunity. We examined CD8+ T cells primed by GAP immunization in wild-type or MHC II-deficient mice. CD8+ T cells primed in the presence of CD4+ T cells showed significantly higher levels of activation than those primed in MHC II-deficient hosts, indicating that CD4+ T cells may be involved in the early priming and expansion phases of the CD8+ memory T cell response. We have created a novel GAP P. yoelii strain that expresses ovalbumin and can be used in combination with ovalbumin-specific transgenic T cells to study how an effective CD8+ memory T cell population can be induced. Through immunization-challenge studies with this vaccine strain in bone marrow-chimeric mice we can also determine which cell populations in the liver are required for priming protective T cells. A better understanding of how parasite antigen is presented and how CD8+ T cells are primed against liver stage infection will inform the development of more powerful anti-malaria vaccines.

Presenter: Näslund, Tanja I.

Exosomes as cancer vaccines and their role in immune regulation

Tanja I. Näslund, Gehrmann U, Qazi K, Karlsson MC and Gabrielsson S

Karolinska Institutet

Antigen presenting cells (APCs), among other cell types, release small (30-100nm) vesicles, so called exosomes. Exosomes from dendritic cells (DCs), which are potent APCs, express MHC class I and II as well as co-stimulatory molecules and can induce both CD4+ and CD8+ T cell responses. Recently, promising results from phase I studies in cancer patients have been reported using exosomes loaded with tumor peptides. However, the physiological role and mechanism of action of exosomes in vivo is still largely unknown. In mice, exosomes derived from bone marrow derived DCs (BMDCs) have been found to stimulate CD4+ T cells in a B cell dependent manner. We have found that CD8+ T cells, a cell type that is highly important in tumor regression, are completely dependent on CD4+ T cells after exosome injection, where no antigen-specific CD8+ T cells could be detected in CD4-/- mice by pentamer staining or IFNg ELISpot assay. Lower levels of antigen-specific CD8+ T cells were also detected in mice lacking B cells, indicating that B cells play a role in exosome induced CD8+ T cell responses. Moreover, in mice lacking Marginal Zone B (MZB) cells (CD19-/- mice) lower levels of antigen-specific CD8+ T cell responses were induced after exosome injection. In conclusion, this study highlight important questions regarding optimization of exosomes as potential cancer vaccines as well as the role of B cells in tumor regression, a cell type largely neglected in cancer vaccine settings.

Presenter: Nguyen, Ngan

Finding disease-associated clonal expansions for ankylosing spondylitis by massively parallel V-D-J sequencing.

Ngan Nguyen, Hyunsung Kim, Maximillian Haeussler, Martha Zuniga, Nader Pourmand, David Haussler

UC Santa Cruz

Ankylosing spondylitis (AS), a form of spondyloarthritis, is a chronic, progressive, connective tissue disorder that is characterized by inflammation of the spinal and sacroiliac joints, affecting approximately 0.1-1.0% of the world population. In this study, we investigate the T-cell receptor beta chain (TCRB) repertoire of CD8+ T cells in the peripheral blood of AS patients and healthy individuals to detect and characterize AS-specific TCRs. We have constructed and sequenced TCRB mRNA and DNA for 5 AS cases and 1 control with a total of more than 100 millions reads and identified approximately 159000 distinct TCRB sequences across all samples. Among these sequences, 2238 were previously reported in the IMGT (ImMunoGenetics) database, including sequences reported from studies of arthritis, e.g reactive arthritis and AS. 85 of the 2238 sequences were expanded in at least two of the cases and not the control. The results show that profiling and comparing case and control TCR repertoires is in the right direction towards uncovering the disease pathogenesis of AS and immune disorders in general.

Presenter: Nguyen, Trang T.

Induction of B cell proliferation by BCR-crosslinking requires the presence of secreted IgM

Trang T. Nguyen, Rebecca A. Elsner and N. Baumgarth

Graduate Group in Immunology and Center for Comparative Medicine, University of California, Davis.

B cells express a surprising number of receptors able to bind secreted (s) IgM, but with seemingly different outcomes. Binding of sIgM via the complement receptors CR1/2 might increase B cell receptor (BCR)-mediated signals, while binding via the Fcalpha/muR appears to act as inhibitor of B cell activation. However, the precise functions of these and other sIgM receptors for B cell response development are largely unknown. This study aims to elucidate the role of sIgM in B cell activation. For that we used secreted IgM knock out mice (us-/- mice), which lack the splice-region for secreted IgM but still express the membrane-bound form. Thus they have normal B cell development and their B cells are able to secrete downstream antibody isotypes. However, as we show here, MACS-purified B cells from us-/- mice show a profound proliferation defect in response to anti-IgM (Fab)2 stimulation in vitro, compared to B cells from wildtype mice. Adoptive transfer of us-/- B cells into Ig-allotype disparate wildtype mice for 48h resulted in increased binding of sIgM onto the surface of us-/- B cells, as determined by flow cytometry. Following their re-isolation, these B cells now proliferated strongly in response to anti-IgM stimulation. Depletion of complement component 3b by administration of cobra venom factor prior to adoptive transfer of the B cells had little effect on surface IgM binding and no effect on anti-IgM mediated B cell proliferation, suggesting that complement-mediated IgM binding was not responsible for mediating co-stimulatory signals. Taken together, our data show that sIgM enhances BCR-mediated proliferation and they indicate that sIgM-binding via an FcuR might be a crucial co-stimulatory signal for B cell clonal expansion. Supported in part by NIH AI51354 and a fellowship from the Vietnamese Education Foundation (to T.T.N).

Presenter: O'Hara Hall, Aisling

A role for IL-27 in the development of T-bet+ Treg required to limit infection-induced pathology

Aisling O'Hara Hall, Cristina Tato (2), Guillaume Oldenhove (3), Beena John (1), Daniel P. Beiting (4), Claudia Gonzalez Lombana (1), Laurence A. Turka (5), Steven L. Reiner (6), Daniel Cua (2), Yasmine Belkaid (3), M. Merle Elloso (7), and Christopher A. Hunter (1),

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Presenter: Ou, Jing-Ni

PD-L1 as a Marker of Renal Disease in Systemic Lupus Erythematosus (SLE)

Jing-Ni Ou, Ou, J-N. and Stevens, A.M.

Seattle Children's Research Institute

Title: PD-L1 as a Marker of Renal Disease in Systemic Lupus Erythematosus (SLE) Ou, J-N. and Stevens, A.M. ABSTRACT Background: Monocytes and dendritic cells from SLE patients display aberrant phenotypes, namely abnormal cytokine production, defective phagocytosis of apoptotic cells, and hyperstimulatory activity for allogeneic CD4+ T cells. We found a diminutive induction of programmed death ligand-1 (PD-L1) on monocytes and myeloid DCs from children with active SLE. Some patients regained PD-L1 expression during disease remission. Objective. To test for a correlation between PD-L1 on monocytes and renal disease in SLE patients. Methods. We phenotyped expression of PD-L1 in SLE patients with class III or IV lupus nephritis (10 children with active disease, 11 in remission); 31 children with SLE without renal disease (10 with active disease, 21 in remission) and 28 age-matched healthy controls. Expression of PD-L1 protein and mRNA was determined in peripheral blood mononuclear cells (PBMC) or isolated CD14+ monocytes by multiparametric flow cytometry and quantitative real-time RT-PCR. SLE disease activity was determined by the SLE disease activity index (SLEDAI), where active disease was defined by a SLEDAI >4. Results. Expression of PD-L1 correlated with SLE disease activity in patients without lupus nephritis ($p = 0.03$). In contrast, expression of PD-L1 protein on monocytes remained low in children with class III or IV lupus nephritis even during remission, and did not correlate with disease activity ($p = 0.17$). Patients with lupus nephritis had decreased expression of PD-L1 surface protein on monocytes compared to patients without nephritis and healthy controls. Significant suppression of PD-L1 mRNA was found in most children with and without lupus nephritis regardless disease activities compared to healthy controls. Conclusion. PD-L1 protein expression inversely correlated with SLE disease activity in the absence of renal disease. Children with class III or IV lupus nephritis retained low expression of PD-L1 protein and mRNA, suggesting deficient expression of PD-L1 may associate with lupus nephritis in pediatric SLE.

Presenter: Paley, Michael A.

T-box transcription factors coordinate terminal differentiation and population renewal in CD8 T cells during chronic viral infection

Michael A. Paley, Scott M. Gordon, Steve L. Reiner, E. John Wherry

University of Pennsylvania, Philadelphia, PA

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Presenter: Pelletier, Adam-Nicolas

The size of the plasmacytoid dendritic cell compartment is a multigenic trait dominated by a locus on mouse chromosome 7

Adam-Nicolas Pelletier, Fanny-Guimont-Desrochers (1,2), Michelle Ashton (3), Tom Brodnicki (3), Sylvie Lesage (1,2)

1. Universit_ de Montr_al, Canada; 2. Maisonneuve-Rosemont Hospital Research Center, Montr_al, Canada; 3. St-Vincent's Institute of Medical Research, Fitzroy, Australia

Plasmacytoid dendritic cells (pDC) compose one of the many distinct dendritic cell (DC) subsets. The primary function of pDC is to potently produce type 1 interferons upon stimulation, which is highly relevant in anti-viral responses. Consequently, the ability to manipulate the size of the pDC compartment in vivo may increase the capacity to clear viral infections. In an attempt to identify genetic loci affecting the size of the pDC compartment, defined by both the proportion and absolute number of pDC, we undertook an unbiased genetic approach. Linkage analysis using inbred mouse strains identified a locus on chromosome 7 (Pdcc1) significantly linked to both the proportion and the absolute number of pDC in the spleen. Moreover, loci on either chromosome 11 (Pdcc2) or 9 (Pdcc3) modified the effect of Pdcc1 on chromosome 7 for the proportion and the absolute number of pDC, respectively. Further analysis using mice congenic for chromosome 7 confirmed Pdcc1, demonstrating that variation within this genetic interval can regulate the size of the pDC compartment. Finally, mixed bone marrow chimera experiments showed that both the proportion and the absolute number of pDC are regulated by cell- intrinsic hematopoietic factors. Our findings highlight the multigenic regulation of the size of the pDC compartment and will facilitate the identification of genes linked to this trait.

Presenter: Prasad, Sharanya

Presentation of cryptic peptides by MHC I molecules is enhanced in virus infected cells

Sharanya Prasad, Shelley Starck, Nilabh Shastri

University of California, Berkeley

Cytolytic T cells eliminate infected cells by recognizing pathogen-specific peptides presented by MHC class I molecules. The antigenic peptides are derived primarily from newly synthesized proteins including those produced by cryptic translation. Previous studies have shown that initiation of cryptic translation can occur at non-AUG codons rather than the canonical AUG initiation codon. Furthermore, CUG-initiated translation is mechanistically distinct from AUG-initiated translation as it is resistant to canonical protein synthesis inhibitors that otherwise cause global translation shutdown. Here, we show that signaling pathways involved in pathogen recognition also enhance presentation of the cryptic peptide in-vitro and ex-vivo using several Toll-like receptor ligands. Moreover, infection of bone-marrow derived macrophages with MCMV and Influenza also specifically boosts the presentation of the cryptic peptide. Thus, translation and presentation of crvptic peptides may allow the immune system to detect intracellular pathogens that inhibit host translation and presentation of pathogen-specific peptides from conventional sources.

Presenter: Raubitscheck, Antony

Insights into transcriptional regulatory networks from genome-wide identification of cis-Regulatory elements in human CD4 T cell subsets

Antony Raubitscheck, Shane Neph, Theresa K Canfield, Chris Wilson, John Stamatoyannopoulos, Steve Ziegler

University of Washington

Recent evidence suggests a degree of plasticity whereby T cells of a given subset are able to express cytokines characteristic of other T cell subsets. We hypothesize that an important underlying mechanism for maintenance of specific effector subsets, as well as any flexibility in these subsets and their phenotype, is epigenetic regulation of the transcriptional regulatory network composed of transcription factor interactions with cis-regulatory elements. By isolating helper T cell subsets from the blood and expanding them in vitro in conditions which support the maintenance of the various subsets, we obtained phenotypically pure naive CD4, Th1, Th2, Th17, and Treg subset populations. From these subsets we isolated nuclei, treated with DNase I, purified fragments via a density gradient, and performed massively parallel sequencing on the resulting DNA fragments. This approach directly identifies regions of DNA which are interacting with transcription factors in a non-biased, genome-wide manner. Global analysis of purified T cell subsets has identified approximately 80,000 regions that show differences in DNase I sensitivity between subsets. We have identified numerous regions in proximity to cytokine, chemokine receptor, and lineage-associated transcription factor genes. These regions represent specific genomic regions, which interact with transcription factors in order to regulate gene expression, of which only a small fraction have undergone any previous characterization. Many of these regions show characteristic

Presenter: Renkema, Kristin

The aging CD8 T cell response: Evaluating and fixing T-bet

Kristin Renkema, Emily Goldberg, Megan J. Smithey, Janko Nikolich-Zugich
University of Arizona

Today, people aged 65 years and older number about 33 million in the United States; this age group is projected to more than double by 2030. Alarmingly, infectious diseases remain amongst the leading causes for death in people over 65 years old. Widespread defects in the immune system have been found to increase with age, led by marked qualitative and quantitative decline in adaptive immunity. CD8 T cells have also been found to decrease in both number and function in aged mice. We recently found that in response to systemic Listeria monocytogenes (Lm) infection, old mice mobilize fewer Lm-specific CD8 T cells. Moreover, these cells exhibited lower effector molecule production, and are less polyfunctional. Previously we evaluated the expression of the key transcription factor T-bet, which is crucial to effector CD8 T cell development and found it was reduced in old CD8 T cells, correlating with lower expression of effector molecules. Now we found that altered transcriptional activation may constitute a common denominator underlying suboptimal function of old CD8 T cells. We have used Interleukin-12 (IL-12) administration to old mice as well as direct T-bet transduction into old CD8 T cells as strategies to improve old CD8 T cell effector development and will report on their relative efficacy in restoring differentiation.

Presenter: Resop, Rachel

Characterizing the dynamics of shingosine-1-phosphate mediated egress of na_ve mature thymocytes from the human thymus

Rachel Resop, Marc Douaisi, Josh Craft and Christel Uittenbogaart

UCLA

Shingosine-1-phosphate (S1P) is a chemotactic sphingolipid molecule which plays an important role in the chemotaxis of a variety of cell types, including thymocytes, and is widely present systemically. We investigated the dynamics of S1P and its five known receptors, S1PR 1-5, in the context of mature na_ve thymocyte egress from the human thymus to the periphery, which is thus far not understood. We hypothesized that thymocytes migrate toward S1P when ligated to S1PR1, 3, 4 and 5 but that S1PR2-S1P ligation promotes repulsion. To examine the kinetics of migration in response to S1P, we performed migration assays in transwell-membrane plates with various concentrations of S1P and additionally several agonists and antagonists to S1P (FTY720, a S1PR1, 3, 4 and 5 receptor antagonist, JTE-013, a specific S1P2 agonist and W146, a specific S1P1 agonist). Preliminary results confirm that thymocytes respond to S1P and that FTY720 does indeed inhibit migration by inhibiting one or more receptors. Further experiments need to be done to better elucidate the roles of S1PR1 and S1PR2. Additionally we performed real-time PCR to verify the expression of S1PR1-2 on thymocytes and are currently optimizing the procedure. We anticipate that on mature na_ve thymocyte subsets (CD3+CD69-CD62L+CD45RA+) S1PR1 and possibly S1PR3-5 will be upregulated, whereas S1PR2 will be downregulated.

Presenter: Roan, Florence

TSLP influences cytokine levels but not histological inflammation in a model of chronic colitis

Florence Roan, Piper M. Treuting, Steven F. Ziegler

Benaroya Research Institute

Thymic stromal lymphopoietin (TSLP) is a member of the IL-2 cytokine family that has emerged as a central player in Th2-type inflammation. Its role in modulating Th1/Th17 responses is just beginning to be explored. Intestinal epithelial TSLP expression has been shown to be decreased in uninflamed tissue in Crohns disease, suggesting a role for TSLP in regulating immune homeostasis in the gut. Indeed, in mice, lack of TSLP signaling worsened disease severity in an acute, innate immune-mediated model of colitis using dextran sulfate sodium (DSS). We explore whether TSLP modulates inflammation in a CD45RBhi T cell transfer model of chronic colitis. T cells lacking the TSLP receptor (TSLPR) induced equivalent levels of weight loss and colonic inflammation as wild-type cells when transferred into RAG-deficient (RAG KO) mice. TSLP responsiveness in the host was also not required to induce colitis. While RAG KO hosts lacking TSLPR had higher Th1/Th17 cytokine levels, histological colitis scores were not significantly different from RAG KO mice with intact TSLP signaling. These data suggest that in a chronic colitis model, TSLP can influence the cytokine milieu, but that other factors may dominate in determining the clinical outcome. Further research is required to determine whether the role of TSLP in the gut may differ in acute versus chronic inflammatory states.

Presenter: Rockwell, Cheryl

Th2 skewing by activation of Nrf2 in CD4+ T cells

Cheryl Rockwell, Mingcai Zhang, Patrick E. Fields, Curtis D. Klaassen
Michigan State University

Nuclear erythroid 2 related factor 2 (Nrf2) is a transcription factor activated by cell stress, including oxidative and electrophilic stimuli, resulting in the upregulation of a battery of cytoprotective genes. Although best known for its role in detoxication, Nrf2 has also been shown to have a number of anti-inflammatory effects. The role of Nrf2 in T cells is largely unknown, however. Nrf2 can be activated experimentally by a number of different pharmacological agents, including tert-butylhydroquinone (tBHQ), which is also used commerically as a food preservative. The present studies found that the Nrf2 activator, tBHQ, suppresses production of IFNγ in activated CD4+ T cells, while concurrently inducing production of IL-4, IL-5, and IL-13. These effects were also observed in CD4+ T cells that were stimulated with anti-CD3/anti-CD28 in the presence tBHQ for 5 days and then restimulated without further addition of Nrf2 activators, suggesting that the effects are due to CD4+ T cell differentiation. Furthermore, the current studies suggest that activation of Nrf2 diminishes T-bet binding activity, while concurrently enhancing GATA-3 binding activity. Collectively, the present studies suggest that activation of Nrf2 has a suppressive effect on Th1 differentiation, while promoting Th2 differentiation, which likely occurs through inhibition of T-bet activity and potentiation of GATA-3 activity. (This work was funded by NIH grants: ES018885, ES007079 and DK081461.)

Presenter: Rohrbach, Amanda

Biochemical Activation of PAD4 and Implications in Autoimmune Disease

Amanda Rohrbach, Sanja Arandjelovic, Saskia Hemmers, and Kerri Mowen

The Scripps Research Institute

Peptidyl Arginine Demininase 4 (PAD4) converts the amino acid arginine to citrulline in protein substrates in a process known as deimination. PAD4 is critical for the full activation of neutrophils and thus for innate immunity. PAD4 has also been implicated in the pathogenesis of several diseases, including rheumatoid arthritis (RA), where a majority of patients produce autoantibodies to citrullinated proteins. PAD4 can be activated through chemokine and toll-like receptors, leading to histone citrullination and formation of extracellular traps; however, the signaling cascades that activate PAD4 are unknown. Herein we aim to map the biochemical pathways leading to PAD4induced citrullination through TLR4. Additionally, the contribution of PAD4 to antibody-induced arthritis will be assessed using the K/BxN model of RA. Deciphering how PAD4 is regulated and the degree to which its activity contributes to RA pathogenesis is pertinent to understanding innate immune activation and the role of PAD4 in autoimmune diseases.

Presenter: Saunderson, Sarah

The presentation of exosomal antigen to T cells via CD169 macrophages

Sarah Saunderson, P R Crocker, A D McLellan

University of Otago

Exosomes are lipid bound nanovesicles released following fusion of the endosomal limiting membrane with the plasma membrane. Na_ve B cells release high levels of exosomes in response to T cell help, however their role in immunity is largely undetermined. Our recent work has shown that exosomes target marginal metallophilic macrophages in the spleen, and subcapsular sinus macrophages present in the lymph node via the sialoadhesin molecule CD169 (MOMA-1), a member of the sialic acid binding Ig-like lectin (Siglec) family. We now demonstrate that B cell derived exosomes can provide a source of MHC-peptide that drives antigen specific T cell activation, potentially via CD169+ macrophages. Interestingly, although T cells undergo extensive proliferation, they lack cytotoxic ability. This suggests that CD169 targeted antigen may induce tolerance. We are currently investigating the specific role of marginal metallophilic macrophages and subcapsular sinus macrophages in antigen capture and antigen processing of exosomes using CD169-/- mice.

Presenter: Sedy, John

The Cytomegalovirus Mimic of Herpesvirus Entry Mediator Avoids CD160 and Inhibits IL-2 Signaling through B and T Lymphocyte Attenuator Restricting Natural Killer Cell Function

John Sedy, Vasileios Bekiaris, Wendell Smith, Ivana Tomcova, Paula Norris, Chris Benedict, Dirk Zajonc, Carl Ware

Sanford|Burnham Medical Research Institute

Not to be placed on the website.

Presenter: Shafiani, Shahin

Expansion of foreign antigen-specific natural regulatory T cells during Mycobacterium tuberculosis infection

Shahin Shafiani, Crystal T Dinh, Imran Siddiqui, James M Ertelt, Kate Smigiel, Pawan Sharma, Daniel J Campbell, Sing Sing Way, Kevin B Urdahl

Seattle Biomed

Foxp3+ regulatory T (T reg) cells that develop in the thymus, called natural T reg cells, have a propensity to recognize self-peptide:MHC complexes, but their ability to cross-react to epitope-defined foreign antigens has not been shown. Here we used MHC class II tetramers containing an immunodominant peptide derived from Mycobacterium tuberculosis (Mtb) to show that a sizeable proportion of antigen-specific CD4+ T cells expanding in the lung draining lymph node during early Mtb infection expressed Foxp3. These antigen-specific T reg cells exhibited a highly activated phenotype, including high levels of CTLA-4 expression, but did not produce IFN-γ. Infection of mice following adoptive transfer of congenically marked T reg cells and conventional CD4+ T cells showed that the antigen-specific T reg cells arose from the population of pre-existing T reg cells and were not derived from conventional ESAT-6 (4-17)-specific CD4+ T cells. In addition, ESAT-6 (4-17)-specific T reg cells expressed the transcription factor Helios, whose expression has been linked to thymically-derived natural T reg cells, and possessed a different profile of T cell receptors than effector CD4+ T cells. Our results support a model in which foreign-reactive cells are present in the natural T reg cell population, but their expansion depends on the appropriate inflammatory context, such as occurs during Mtb infection.

Presenter: Silvers, Thomas

Role of APOBEC Family Proteins in Immunoglobulin Class Switch Recombination

Thomas Silvers, Zach Carico, Sophia Sarafova, Shyam Unniraman

Davidson College, Duke University

Activation-induced cytidine deaminase (AID) functions in activated mammalian B cells to vary antibody effector function via deletion of immunoglobulin constant region DNA in the process of class switch recombination (CSR). AID deaminates cytidine to uridine, creating mismatch targets for cellular DNA repair mechanisms. Other deaminases closely related to AID are the APOBEC proteins. APOBEC proteins are expressed in activated B cells as well, however they have no known role in CSR. To evaluate the potential involvement of APOBEC1 and APOBEC2 in CSR we used shRNA to knock down their expression in the CH12 B cell line, which is known to switch from IgM to IgA upon stimulation. FACS analysis of membrane IgA expression revealed no significant change in CSR levels after APOBEC1 and APOBEC2 knockdown, while AID knockdown reduces CSR by 60% as expected.

Presenter: Simkins, Helen

Dissecting the requirement for CD169+ macrophages in CD4+ T cell activation

Helen Simkins, Terri Laufer
University of Pennsylvania

Soluble antigen for presentation to CD4+ T cells accesses the lymph node via two different routes: direct drainage though the lymph or via transport by migratory dendritic cells (DC) that acquire antigen in the periphery. Others have demonstrated CD169+ macrophages, which localized to the subcapsular sinus, are important in capturing and presenting soluble antigen to B cells and NKT cells. Furthermore, in conjunction with DC, CD169+ macrophages have been shown to play a part in CD8+ T cell activation during viral infection. We propose to investigate whether there is a requirement for CD169+ macrophages in the transfer of soluble antigen to lymphoid-resident DC resulting in proliferation and activation of CD4+ T cells. To examine this, we used a mouse model whereby CD169+ macrophages are depleted through diphtheria toxin treatment. Results from these studies will be presented. By understanding the role of each antigen presenting cell in CD4+ T cell activation, we can alter the activation process, which will be important for treating CD4+ T cell mediated diseases.

Presenter: Soe, Katherine

Discovery and characterization of novel kinase activity in coactivator of transcription CIITA

Katherine Soe, Devaiah Ballachanda, Dinah Singer
National Cancer Institute, National Institutes of Health

Not to be placed on the website.

Presenter: Soong, Lynn

IL-7 regulates the expansion of IL-17-producing ?d T cells and antiviral responses in mouse liver

Lynn Soong, Lifei Hou, Mayura Desai, Zuliang Jie, Yuejin Liang, Tian Wang, Jiaren Sun
The University of Texas Medical Branch

This study was conducted to study the complex interactions among the liver parenchyma, the innate and adaptive immune components during acute viral hepatitis. Mice were i.v. infected with a recombinant adenovirus. A transient, but significant, accumulation of IL-17-producing cells and IL-17 production was detected in the liver within the first 24 h of infection. In vivo neutralization of IL-17 or IL-23 suppressed the local IL-12 levels and halted the accumulation of cytotoxic T lymphocytes (CTL) and T helper1 (Th1) cells, resulting in alleviation of liver injury. A majority of the IL-17-producing cells in the liver were ?d T cells, belonging to the V?4 subset. Intrahepatic IL-17+ cells, but not the IFN-?+ ones, preferentially expressed IL-7Ra (CD127) on their surface, which coincided with an elevation of hepatocyte-derived IL-7 at 12 h postinfection. IL-7Ra blockade in vivo severely impeded the expansion of IL-17+ cells following viral infection. In vitro, IL-7 synergized with IL-23 and directly stimulated IL-17 production from ?d T cells in response to TCR?d stimulation. Finally, type I interferon (IFN-I) signaling was found to be critical for hepatic IL-7 induction. Conclusion: This study is the first example in which the IFN-I/IL-7/IL-17 axis was shown to play a decisive role in viral hepatitis. The highly coordinated events taking place in hepatocytes, innate and adaptive immune cells eventually led to viral clearance and disease resolution in the liver.

Presenter: Spees, Alanna

The Contribution of CD1d-restricted Natural Killer T cells during Acute Salmonella Typhimurium Infection

Alanna Spees, Ivan Godinez, Mariana Xavier, Renee Tsolis, Andreas Baumler

UC Davis

Natural killer (NK) cells and natural killer T (NKT) cells are important cellular sources of interferon (IFN)-γ. Their contribution of IFN-γ has been shown upon ex vivo stimulation with Salmonella enterica serotype Typhimurium (S. Typhimurium) primarily in systemic tissues. NK and NKT cells share expression of the surface marker NK1.1. Here we investigated the contribution of NK1.1 positive cells and CD1D-restricted NKT cells to intestinal inflammatory responses in vivo using the mouse colitis model of S. Typhimurium acute infection. Interestingly, mice lacking NK1.1 positive cells or CD1D-restricted NKT cells did not exhibit reduced expression of Ifng, the gene encoding IFN-γ, in the cecal mucosa during S. Typhimurium infection as compared to wild type (C57BL/6) mice. The changes in cellular infiltration into cecal tissue may contribute significantly to fluctuations in IFN-γ production during infection, although the major cellular sources have yet to be determined. Importantly, the lack of NK1.1 positive cells or CD1D-restricted NKT cells significantly blunted mucosal expression of Il17a, the gene encoding interleukin (IL)-17A. Although NK1.1 positive cells and CD1D-restricted NKT cells may be major contributors to IFNγ in systemic tissues, these results suggest that NK1.1 mainly function in IL-17A production during S. Typhimurium-induced colitis.

Presenter: Srivastava, Smita

Cell-to-cell transfer of a bacterial antigen contributes to initiation [and evasion] of CD4 T cell activation in tuberculosis.

Smita Srivastava, Joel D.Ernst

New York University School Of Medicine,550 First avenue New York, NY 10016.

Mycobacterium tuberculosis survives in vivo despite the induction of adaptive immune responses. We have previously found that initiation of M. tuberculosis-specific CD4 T cell immunity requires bacterial transport by CD11bhi lung DCs to mediastinal lymph nodes (MLN). While CD11bhi DCs are the most heavily infected cells in the MLN, they are less efficient at stimulating antigen-specific CD4 T cells than other DC subsets after addition of exogenous antigenic peptide. This suggests that they are not the cells that directly prime na_ve CD4 T cells after infection with M. tuberculosis. We found that intra-tracheal transfer of M. tuberculosis-infected MHC-II-/- migratory DC into MHC-II+/+ mice resulted in activation of naive TCR transgenic CD4+ T cells specific for a secreted M. tuberculosis antigen (Ag85B) in MLN, indicating that migratory DCs can transfer a bacterial antigen to other DCs. In vitro studies revealed that M. tuberculosis-infected bone marrow-derived DCs secrete Ag85B via a vesicular pathway that is independent of recycling endosomes, exosomes and/or apoptotic vesicles. In addition, immuno-fluorescence staining of frozen lung sections obtained from M. tuberculosis- infected mice show Ag85+ staining in infected cells as well as in bystander uninfected lung cells. After infection of mice with GFP-expressing M. tuberculosis and flow sorting, uninfected CD11b+CD11chi lung cells activated Ag85B-specific CD4+ T cells as efficiently as did infected cells without addition of exogenous peptide, confirming that uninfected cells acquired, processed, and presented antigen at the site of infection in the lungs. Taken together our results provide direct evidence that M. tuberculosis-infected DCs transfer antigen to uninfected cells in vitro and in vivo in lymph nodes and lungs. These results suggest that transfer of antigen to uninfected cells may allow M. tuberculosis to distract Ag-specific CD4+ effector T cells and thereby persist in the lungs. Therefore, cell-cell transfer of antigen may both promote activation of na_ve CD4+ T cells and evasion of effector CD4+ T cells during chronic infection.

Presenter: Srivastava, Shivani

Type I IFN Directly Inhibits Treg Activity During Acute Viral Infection

Shivani Srivastava, Meghan A. Koch, Daniel J. Campbell

University of Washington

Regulatory T cells (Tregs) are a unique cell population capable of restraining immune responses to both foreign and self-antigens. During viral infection, Treg activity must be tightly controlled such that an immune response is mounted effectively but is promptly terminated upon viral clearance. Type I interferons (IFN) are a group of cytokines that coordinately regulate many cell types during viral infection, but their effects on Tregs remain largely unknown. We found that IFN_ directly inhibited IL-2-dependent Treg proliferation in vitro, and IFN_-treated Tregs were impaired in their ability to suppress effector T cell proliferation. Additionally, Treg cell numbers, proliferation, and activation were significantly reduced early during acute infection with lymphocytic choriomeningitis virus (LCMV), and this inhibition correlated tightly with elevated expression of IFN. IFN directly inhibited Treg activation in vivo, as IFNaR-/- Tregs proliferated more robustly and showed increased activation compared to WT Tregs in WT:IFNaR-/- chimeric mice infected with LCMV. To test whether IFN-mediated inhibition of Tregs was necessary for immunity to LCMV, we infected Foxp3-Cre;Stat1 flox/flox mice, in which Foxp3+ Tregs selectively lack expression of Stat1 and thus are unresponsive to type I and II IFNs. Expansion of virus-specific CD8+ and CD4+ T cells was significantly impaired in Foxp3-Cre;Stat1flox/flox mice, and this was associated with increased Treg cell numbers and activation. These data suggest that IFN directly inhibits Treg activity during viral infection and that IFN-mediated inhibition of Tregs is necessary for expansion of virus-specific effector T cells.

Presenter: Sternberg, Luise

INOSITOL-TRISPHOSPATE 3-KINASE B CONTROLS THE KINETICS OF T CELL DEVELOPMENT BY LIMITING PI3K SIGNALING AND METABOLIC RATE

Luise Sternberg, Yina Huang, Sabine Siegemund, Stephanie Rigaud, Karsten Sauer

The SCRIPPS Research Institute

By recognizing pathogen-derived antigens through T cell receptors (TCR), T cells govern the immune response. They are essential for fighting infections and cancer. To allow recognition of a broad pathogen repertoire, successive somatic TCR B and then A subunit gene recombination in developing thymocytes generates T cells with random TCR specificity. Many of these TCRs don't function. Some are autoreactive and might cause autoimmune disease. To warrant the generation of a functional but self-tolerant T cell repertoire, TCR functionality is assessed at various developmental checkpoints. First, B-selection assesses surface-expression of a preTCR composed of a sucessfully rearranged TCR B chain bound to an invariant pTA chain. Signals from this pre-TCR trigger thymocyte proliferation, survival, TCR A chain rearrangement and progression to the CD4+CD8+ stage. Here, successful TCR A chain rearrangement causes expression of a mature TCR. Its functionality is then assessed through MHC/peptide interactions. Appropriate TCR signaling triggers survival and maturation through positive selection. Inappropriate TCR signaling results in death by 'neglect' or negative selection. Phosphoinositide 3-kinase (PI3K) is an essential TCR effector whose deregulation can impair T cell development and cause leukemia/lymphoma. PI3K acts by producing the membrane lipid PIP3, which recruits signaling proteins by binding to their pleckstrin homology (PH) domains. Inositol-Trisphosphate 3-Kinase B (ItpkB) phosphorylates the second messenger IP3 into soluble IP4. We previously showed that IP4 acts as a soluble PIP3 analog which controls PH domain interactions with PIP3 and thereby establishes a positive feedback-loop of TCR signaling that is essential for positive selection. ItpkB-/- mice lack IP4 and are severely immunodeficient due to a positive selection block. Here, we show that IP4 has another important function in B-selection. preTCR expressing ItpkB-/- thymocytes show increased signaling via the PI3K effector AKT, normal proliferation and survival but increased metabolic activity and an accelerated progression to the CD4+CD8+ stage. Thus, IP4 controls the kinetics of thymocyte development by limiting the metabolic rate through PI3K antagonism.

Presenter: Su, Laura

The pre-existence of memory phenotype in unprimed human CD4+ T cells

Laura Su, Mark M. Davis
Stanford University

Not to be placed on the website.

Presenter: Swan, Gregory

Identification of a novel positive cis-control element in the Cd4 gene

Gregory Swan, Sophia Sarafova
Davidson College

CD4 helper T cells coordinate the immune response and are highly dependent on the expression of the Cd4 gene for proper development and function. The function of a promoter, an enhancer and a silencer have been well documented and together explain how the Cd4 gene gets turned on in CD4 T cells and off in CD8 T cells. However once turned on, the amount and timing of Cd4 expression varies during T cell development and activation. This modulation of CD4 surface levels is essential for proper lineage specification and T cell function. Yet, how subtle changes of CD4 expression are regulated remains unclear. We have recently identified a novel positive cis-acting transcriptional regulatory element (NCE) in the Cd4 locus. Here we demonstrate that NCE enhances Cd4 promoter function in position and orientation independent manner using a transient transfection assay with an eGFP reporter construct in RLM11 murine thymoma cell line. This formally demonstrates that NCE functions as an enhancer.

Presenter: Swarts, Sarah

THE DIFFERENTIAL ROLE OF IL-17RA IN BRAIN VERSUS SPINAL CORD INFLAMMATION DURING EAE

Sarah Swarts, Denny Liggitt, Joan M. Goverman

University of Washington

Multiple sclerosis (MS) has been studied for decades using an animal model referred to as Experimental Autoimmune Encephalomyelitis (EAE). EAE is induced by stimulating T cell-mediated immunity to myelin proteins. Two subsets of effector T cells have been suggested to be instrumental in the pathogenesis of EAE: TH1 (IFN-γ secreting) and TH17 (IL-17 secreting) cells. In most rodent EAE models, parenchymal inflammation predominantly targets the spinal cord, a pattern that differs from that seen in most MS patients. Our lab has developed an atypical EAE model in which parenchymal inflammation occurs extensively in the brain and the spinal cord. By varying the ratio of myelin-specific TH1 and TH17 cells adoptively transferred into mice, we were able to control whether inflammation occurred in the brain or not. We found that the spinal cord was susceptible to inflammation over a range of TH17:TH1 ratios, while a high TH17:TH1 ratio was required for inflammation in the brain. Therefore, we hypothesize that the brain and spinal cord function as distinct microenvironments that differ in their response to TH1 and TH17 cells, and that this may contribute to the varying patterns of inflammation seen in EAE. To test this hypothesis, we investigated whether the effect of IL-17 differed in the brain compared to the spinal cord by adoptively transferring wild-type TH17-skewed cells into either wild-type or IL-17receptorA-deficient (IL-17RA-/-) recipients. We found that the incidence of classic EAE that typically reflects predominantly spinal cord inflammation was increased in IL-17RA-/- compared to wild-type recipients, suggesting that IL-17RA signaling may preferentially enhance brain inflammation without influencing spinal cord inflammation. We next tested whether IL-17RA-signaling promotes neutrophilic infiltrates in the brain versus the spinal cord. Interestingly, IL-17RA signaling was required for neutrophil recruitment in the brain, but not in the spinal cord, suggesting that this differential requirement for neutrophil recruitment could be responsible for IL-17 mediated brain inflammation. To our surprise, IL-17RA signaling was not required for brain inflammation following active EAE induction, and we are currently investigating the mechanistic basis for the differential requirement for IL-17 in mediating brain inflammation during passive versus active EAE induction. Initial experiments suggest that the lack of IL-17RA signaling on T cells may enhance brain inflammation in IL-17RA-/- animals during active induction, and we are currently investigating potential mechanisms contributing to this phenomenon.

Presenter: Tuladhar, Shraddha

Shaping the Immune Response to Francisella tularensis by the First Cell Type Infected

Shraddha Tuladhar, Lydia Barrigan, Shaun Steele, Lynn Edde, Matthew Woolard, Thomas Kawula, Jeffrey Frelinger

University of Arizona

Francisella tularensis is a facultative, intracellular coccobacillus and the causative agent of tularemia. F. tularensis induces a host response that is dependent on the route of infection. Intranasal (i.n.) inoculations are more virulent and require fewer bacteria to produce a lethal infection than an intradermal (i.d.) inoculation (103 organisms i.n. versus 106 organisms i.d.). Interestingly, at one day post infection, the bacterial loads are similar in the spleen and lung regardless of the route of infection. We also found that i.d. inoculation resulted in IFN-?+ T cells in the lung whereas i.n. inoculation produced very few IFN-?+ T cells and instead many IL-17+ T cells in the lung. Due to the similar bacterial loads in the lungs but very different host responses, we hypothesize that the adaptive immune response is influenced by local events at the site of infection immediately following inoculation. To test this hypothesis, we identified the first cell type infected in the lungs of 6 -10 week old C57Bl6/J mice given intranasal and intradermal inoculations of F. novicida U112, the live vaccine strain (LVS), or the highly virulent SchuS4 strain of F. tularensis using flow cytometry. At four hours post-infection, we found that for all three subspecies of F. tularensis, alveolar macrophages are the primary cell type infected, to the exclusion of other myeloid cells and lung parenchyma. After seventy-two hours post i.d. infection interstitial macrophages and neutrophils are the primary cell types infected in the lungs. Furthermore, we have depleted alveolar macrophages from the CD11cDTR mice which have diphtheria toxin receptor on CD11c promoter which are expressed at higher levels in alveolar macrophages. At four hours post i.n. infection, CD11cDTR mice which have alveolar macrophages depleted have fewer infected cells and a lower bacterial burden than the C57BI6/J mice. This would help us understand the subsequent immune response to F. tularensis in the absence of alveolar macrophages.

Presenter: Umiker, Benjamin

Aicda expression in immature B cells as a potential mechanism for tolerance and autoantibody production in a model of systemic lupus erythematosus (SLE)

Benjamin Umiker, Amma Larbi, Tasuku Honjo, Thereza Imanishi-Kari.

Tufts Sackler School

Systemic lupus erythematosus (SLE) is a debilitating autoimmune disorder, which is characterized by anti-nuclear antibody production. Our work aims to understand the mechanisms by which B cells producing autoantibodies avoid tolerance and cause disease. Our main hypothesis is that activation induced deaminase (AID) expression in immature B cells by means of somatic hyper-mutation (SHM) generates a mechanism of tolerance against auto-reactive antibodies. In addition, class switch recombination (CSR) in immature B cells is a mechanism for avoiding tolerance and therefore a driver of self-reactivity. To investigate these ideas we use a model of SLE termed 564 Igi._564 Igi has a heavy and light chain double knock-in, which codes for a nucleic acid specific auto-antibody. This mouse model is highly reminiscent of SLE in humans._On a 564 Igi and Aicda knockout background we were able to conditionally express a floxed AID transgene in all developing B cells in one mouse model (CD19-cre) and in only mature B cells in another model (CD21-cre). As of today we have found that 100% of CD19-cre express anti-RNA IgG antibodies. On the other hand, only 12% of CD21-cre mice have anti-RNA IgG antibodies in their sera. These findings support the idea that a majority of the auto-reactive IgG antibodies are produced in the immature B cell stages. This is an important finding because it suggests CSR as a mechanism to avoid tolerance that may be a crucial step in the development of SLE pathophysiology. All sera which produce anti-RNA IgG antibodies bind strongly to an antibody made against the idiotype (Id+) of the 564 transgene. Interestingly, there is a population (38%) of CD21-cre mice with Id+ antibodies but have no IgG anti-RNA binding. This suggests that in CD21-cre mice there is a population of tolerized B cells expressing somatically mutated antibody genes. We conclude that pathogenic autoantibodies in our mouse model of SLE are produced during early B cell development.
Acknowledgement: Grants NIH R01AI076409, and LRI

Presenter: Vacchio, Melanie

A critical role for Thpok in maintaining peripheral CD4 T cells

Melanie Vacchio, Lie Wang, Yumei Xiong, Jong Kyong Kim and Remy Bosselut

Laboratory of Immune Cell Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20892

The transcription factor Thpok is critical for commitment of class II-restricted thymocytes to the CD4 lineage. Similarly the transcription factor Runx3 directs CD8 lineage commitment. Expression of either of these two transcription factors acts to inhibit function of the other during thymic development. However, in apparent contrast to its thymic role, Runx3 is not only expressed but functional in mature CD4 Th1 cells despite co-expression of Thpok. This observation reflects how little is understood about the post-thymic interplay between these transcription factors. We generated mice conditionally deficient for Thpok that express cre recombinase under control of a promoter that is upregulated in SP thymocytes (Thpokpd) to eliminate Thpok expression in mature CD4 T cells. We found that post thymic Thpok disruption caused CD8 reexpression by activated CD4 cells. However, CD8 re-expression depended on the cytokine environment and was more pronounced under Th1 culture conditions that promote IFNγ production. Accordingly, CD8 reexpression required the transcription factor Runx3, a critical player of Th1 differentiation. Our results indicate that Thpok separately antagonizes Runx3 and CD8 expression in peripheral T cells and is necessary to maintain separation between peripheral CD4 and CD8 functional responses.

Presenter: Villacres, Maria C.

Immune-regulatory role of IL10 in treated HIV infection

Maria C. Villacres, Naoko Kono, Wendy J. Mack, Marek J. Nowicki, Kathryn Anastos, Michael Augenbraun, Chenglong Liu, Alan Landay, Ruth M. Greenblatt, Stephen Gange and Alexandra M. Levine

University of Southern California

Not to be placed on the website.

Presenter: Volkman, Hannah

Exploring the Connections Between Antiviral Responses and Autoimmunity

Hannah Volkman, Daniel B. Stetson
University of Washington, Seattle

Not to be placed on the website.

Presenter: Whitley, Debbie

Characterizing the contribution of endogenous retroelements in autoimmune disease pathology

Debbie Whitley, Tina Gall, Daniel Stetson
University of Washington

Not to be placed on the website.

Presenter: Whorton, David

The Murine Immune Response to Corynebacterium pseudotuberculosis: A Model for Vaccine Development

David Whorton, Kristina Geiger, Megan Sumida, Elise Burger, Sharon Spier, Dr. Roberta Pollock, Dr. Karen Molinder

Occidental College

Pigeon Fever is an equine disease caused by the gram positive bacteria Corynebacterium pseudotuberculosis. This increasingly frequent disease is characterized by external abscesses (91% of the cases, with a mortality rate of 0.8%), internal abscesses (8% of the cases, with a mortality rate of 30-40%), and a third less common form, ulcerative lymphangitis (1% of the cases). We have developed a mouse model to study the effectiveness of various potential vaccines and to gain more insight into the antibody and cytokine responses to this bacteria. For our first experiment, BALB/c mice were immunized before bacterial challenge with: (1) inactivated phospholipase D (PLD, the major exotoxin of this bacteria), (2) cell lysate (internal components of the bacterial cells), (3) cell debris (cell wall and other insoluble components of the bacterial cells), and (4) concentrated bacterial culture supernatant (proteins secreted from the bacterial cells). These mice were immunized, boosted, and then challenged with 5x10^3 live bacteria. In a second experiment, BALB/c and C57Bl/6 mice were injected with varying amounts of bacteria, ranging from 10^2-10^6 cells/mouse to determine the optimal challenge concentration. Next, in our third experiment, BALB/c and C57Bl/6 mice were immunized and then boosted with formalin-inactivated PLD or a commercial sheep vaccine, targeted against the ovis biovar of the bacteria. These mice were then challenged with 5x10^6 bacteria cells/mouse. The serological response of all mice to either PLD or lysed cells is examined by ELISA analysis of IgG levels over the course of each experiment. A better understanding of the immune response to C. pseudotuberculosis will greatly facilitate the development of a successful equine vaccine.

Presenter: Yang, Cliff

The transcriptional regulators Id2 and Id3 control the formation of distinct memory CD8+ T cell subsets.

Cliff Yang, Yang CY, Best JA, Knell J, Yang E, Sheridan AD, Jesionek AK, Li HS, Rivera RR, Lind KC, DCruz LM, Watowich SS, Murre C, Goldrath AW

University of California, San Diego

During infection, naive CD8+ T cells differentiate into effector cells, which are armed to eliminate pathogens, and memory cells, which are poised to protect against reinfection. The transcriptional program that regulates terminal differentiation into short-lived effector-memory versus long-lived memory cells is not clearly defined. Through the use of mice expressing reporters for the DNA-binding inhibitors Id2 and Id3, we identified Id3hi precursors of long-lived memory cells before the peak of T cell population expansion or upregulation of cell-surface receptors that indicate memory potential. Deficiency in Id2 or Id3 resulted in loss of distinct CD8+ effector and memory populations, which demonstrated unique roles for these inhibitors of E-protein transcription factors. Furthermore, cytokines altered the expression of Id2 and Id3 differently, which provides insight into how external cues influence gene expression.

Presenter: Yong-Min , Choi

Induction of chemokines mediated by TNF-[a] in TRAF6 deficient cells

Choi Yong-Min , Hyuk-Kwon Kwon, Shaherin Basith, Sangdun Choi

Ajou university

Not to be placed on the website.

Presenter: Zhang, Linyi

A new method for the characterization of the immunosuppressive activities of regulatory T cells for preventative and therapeutic interventions in T1D susceptible NOD mice

Linyi Zhang, Jean Manirarora, and Cheng-Hong Wei

Gene Therapy and Immunogenicity Branch, Division of Cellular and Gene Therapy, CBER, FDA, Bethesda, USA 20892

CD4+CD25+FOXP3+ regulatory T cells (T reg cells) play an important role in suppressing other effector T Cells. Current experimental methods in T reg research have several technical challenges, including insufficient number of Treg cells in peripheral blood, requirement for stringent expansion condition, and the lack of antigen-specific system for Tregs to counteract. To overcome those obstacles, we developed a novel, sensitive in vitro assay to measure the immunosuppressive activity of Tregs. The assay uses the NIT-1 pancreatic beta cell line as target cells, and the TCR transgenic 8.3 CD8 T cells as effector T cells to lyse the NIT-1 cell which expresses the specific antigen recognized by 8.3 T cells. We also use the modified Cell Titer-Glo technology from Promega to measure luciferase activity as a global indicator of cytotoxicity. The quantity of luminescent signal is the indicator of the number of viable cells in cell culture. This is a highly sensitive in vitro assay system that can detect the interaction of Tregs with antigen specific CD8+ effector T cells. It demonstrated a broader range of sensitivity, and was able to detect killing of NIT1 cells by 8.3 T cells, as well as the inhibition by regulatory T cells. The luminescent signal showed a linear relationship (R2 = 0.99) with the number of cells from 150 to 40,000 cells. In addition, the system demonstrated the antigen specificity of CD8 8.3 cytotoxicity and inhibitory activities of Tregs under a variety of conditions. The outcome of the assay showed the comparability with CFSE assay, and yet it possesses a superb advantage of using much lower T reg cell number in the assay. Our results using 2K NIT1 per testing well showed that natural Tregs from both NOD and B6 mice can partially inhibit cytotoxic activity of activated 8.3 T cells. Moreover, we found that IL-2/IL-2 mAb complexes can significantly enhance the inhibitory activities of nTregs from both B6 and NOD mice during the priming and effector phases. The inhibitory effects of Tregs from B6 mice are much stronger than that from NOD mice in any condition tested. In summary, our preliminary results demonstrated that this new in vitro method is a very sensitive way to functionally characterize Tregs immunosuppressive activities against islet antigen-specific CD8 effector T cells. (This study was supported by intramural funding from CBER,FDA)